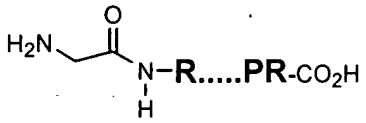
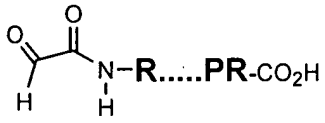


Domain 1 of β_2GPI (D_1, where bold letters stand for single letter amino acid code of terminal amino acids of Domain 1 of β_2GPI)

pH 5.5

1-2 M NaOAc
$CuSO_4$
glyoxylic acid



Transaminated Domain 1 (**TA/D1**)
Comprising a terminal glyoxyl group

Figure 1

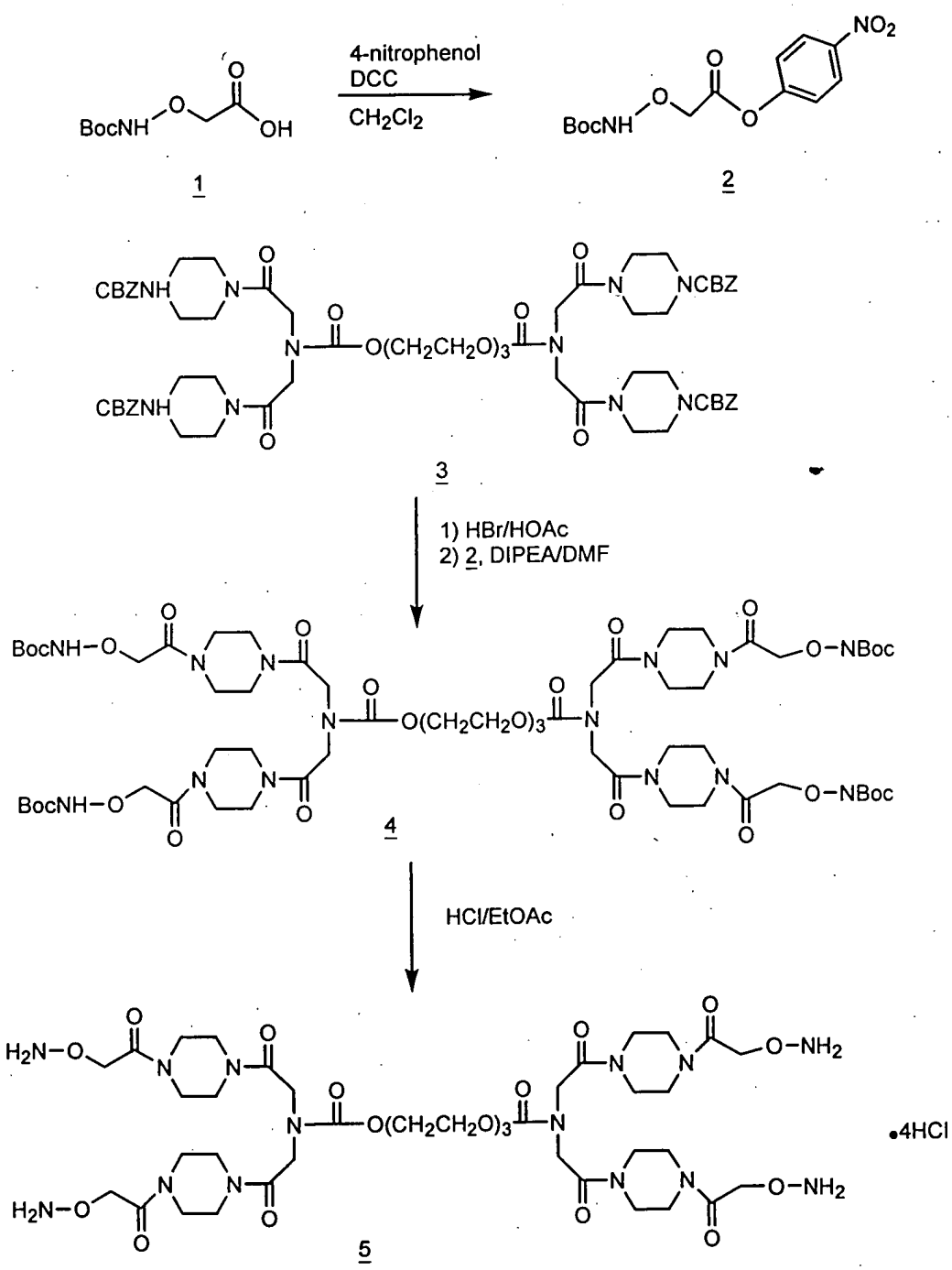

4-nitrophenol
DCC
CH_2Cl_2
BocNH
OH
NO_2
1
2
CBZNH
NCBZ
$O(CH_2CH_2O)_3$
3
1) HBr/HOAc
2) 2, DIPEA/DMF
BocNH–O
O–NBoc
4
HCl/EtOAc
H_2N–O
O–NH_2
•4HCl
5

Figure 2

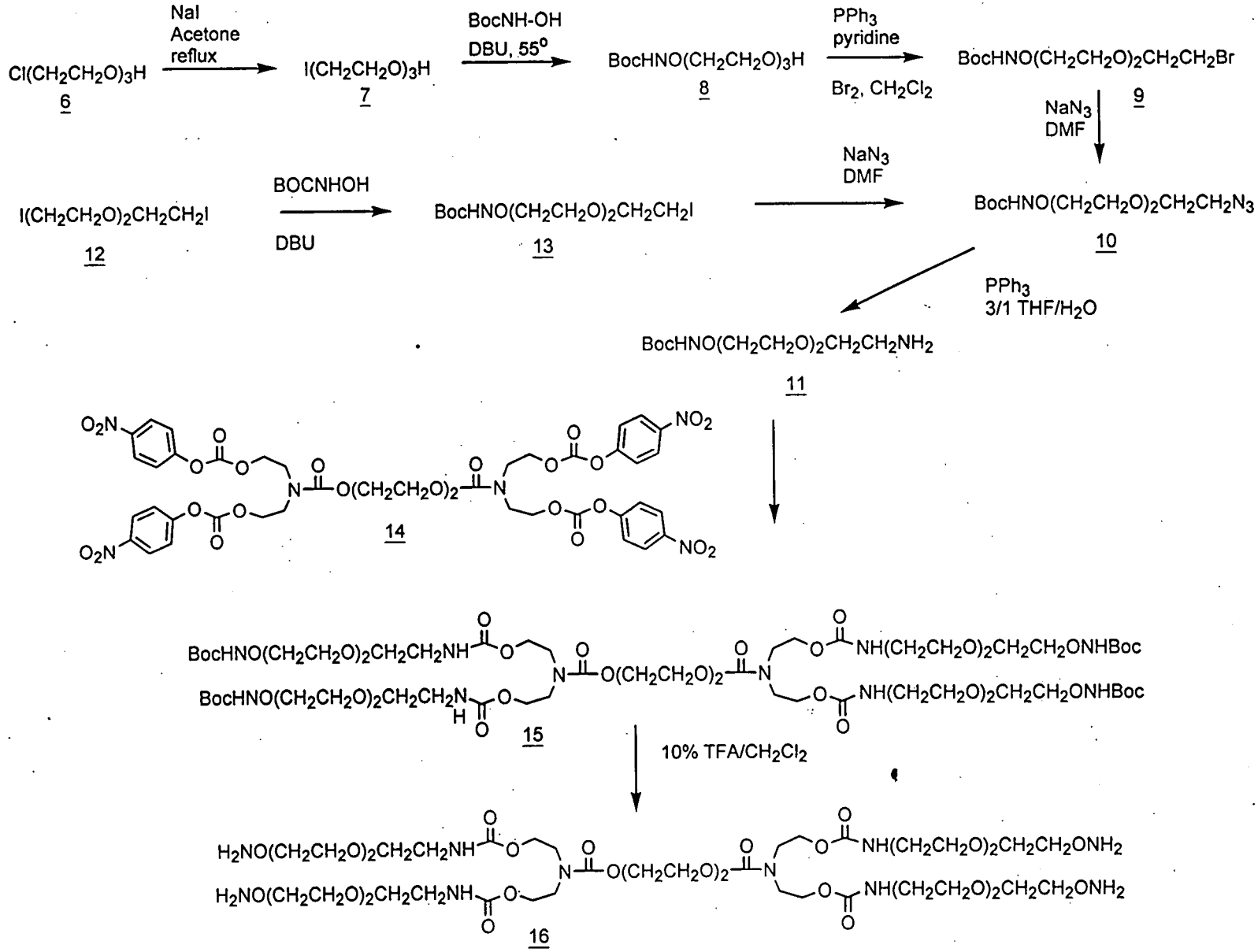


Figure 3

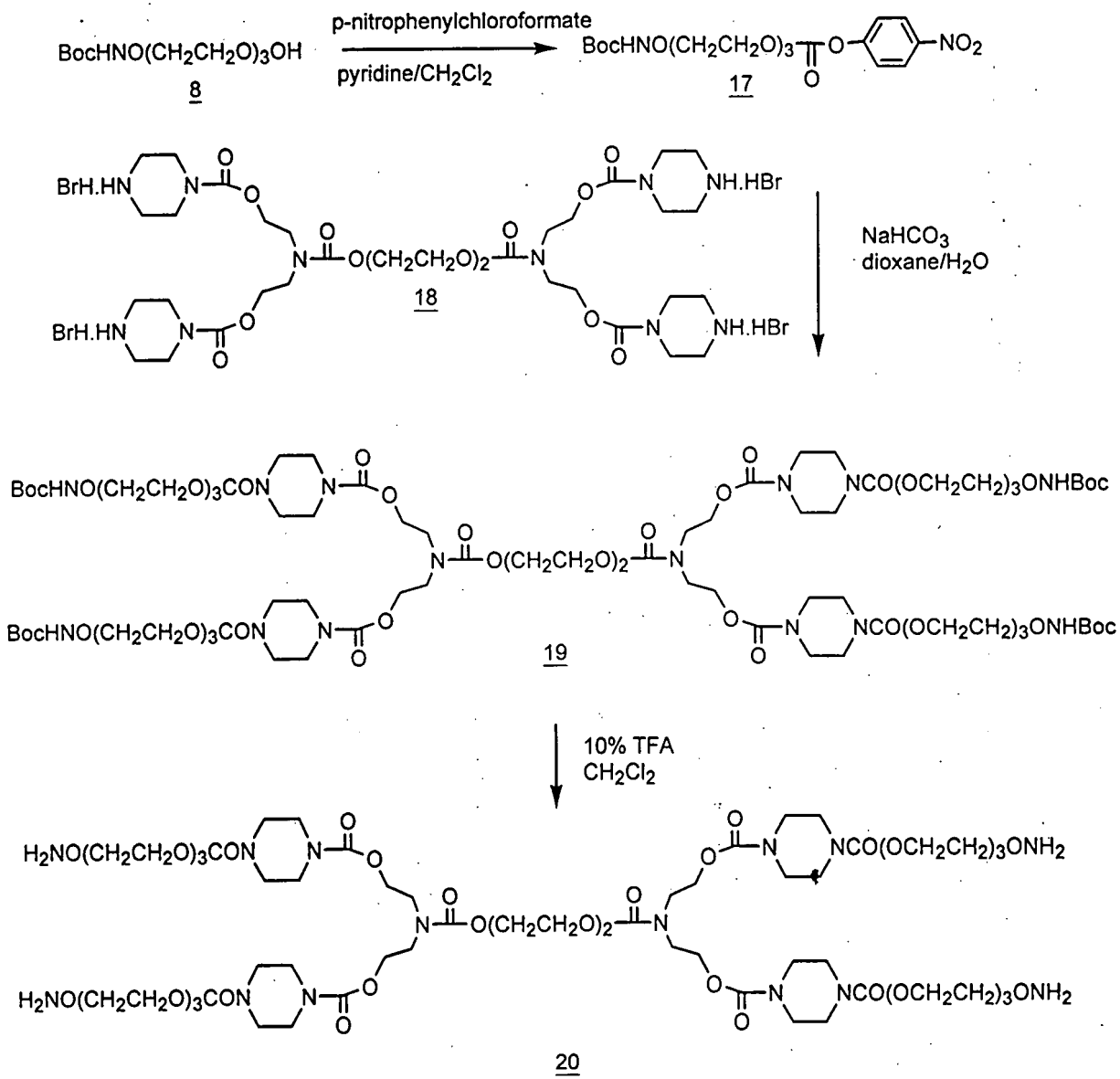

p-nitrophenylchloroformate
pyridine/CH2Cl2
BocHNO(CH2CH2O)3OH
8
BocHNO(CH2CH2O)3
17
NO2
BrH.HN
NH.HBr
O(CH2CH2O)2
18
NaHCO3
dioxane/H2O
BocHNO(CH2CH2O)3CON
NCO(OCH2CH2)3ONHBoc
19
10% TFA
CH2Cl2
H2NO(CH2CH2O)3CON
NCO(OCH2CH2)3ONH2
20

Figure 4

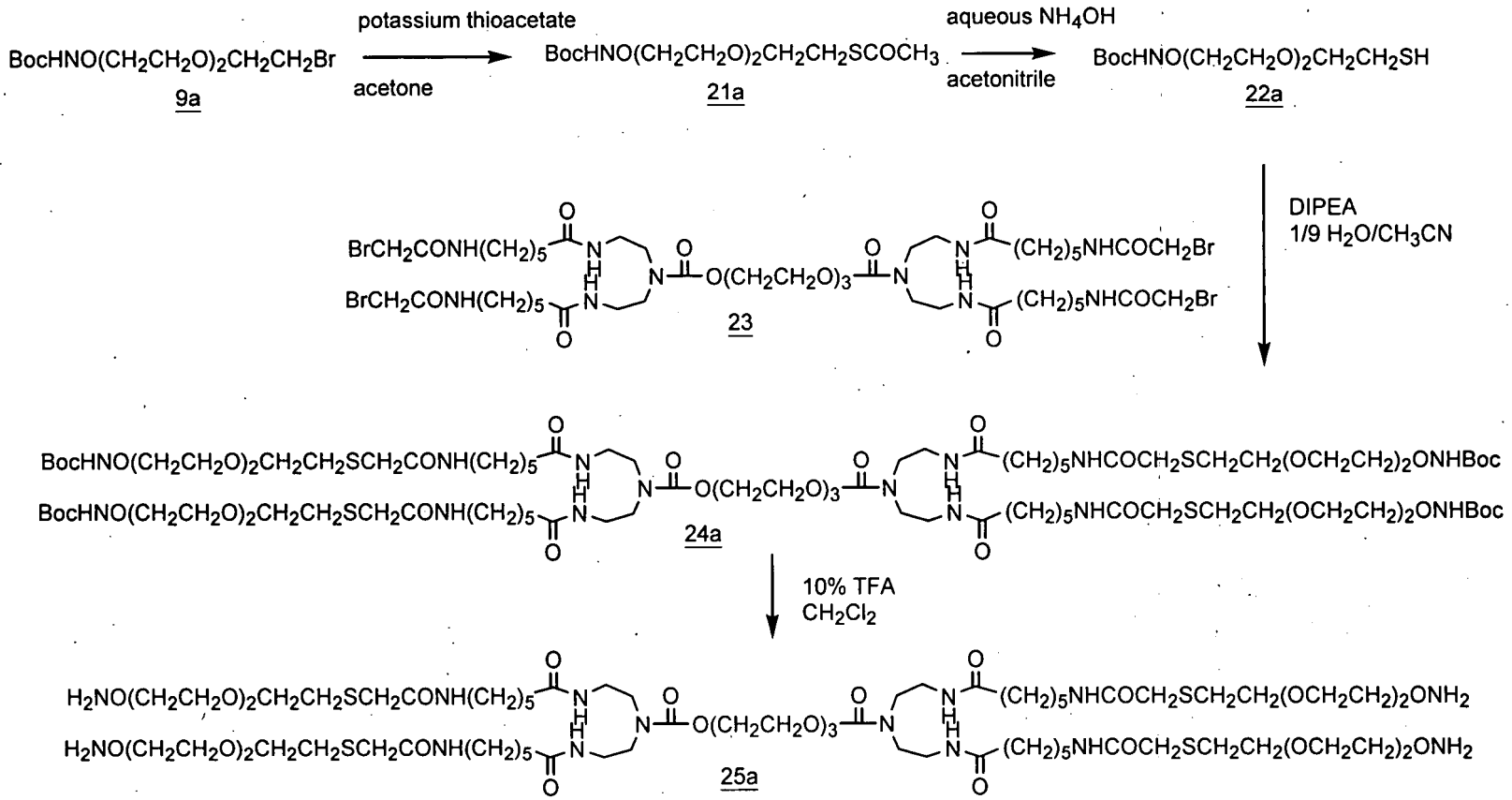

potassium thioacetate
acetone
aqueous NH4OH
acetonitrile
BocHNO(CH2CH2O)2CH2CH2Br
9a
BocHNO(CH2CH2O)2CH2CH2SCOCH3
21a
BocHNO(CH2CH2O)2CH2CH2SH
22a
BrCH2CONH(CH2)5
O(CH2CH2O)3
(CH2)5NHCOCH2Br
23
DIPEA
1/9 H2O/CH3CN
BocHNO(CH2CH2O)2CH2CH2SCH2CONH(CH2)5
(CH2)5NHCOCH2SCH2CH2(OCH2CH2)2ONHBoc
24a
10% TFA
CH2Cl2
H2NO(CH2CH2O)2CH2CH2SCH2CONH(CH2)5
(CH2)5NHCOCH2SCH2CH2(OCH2CH2)2ONH2
25a

Figure 5

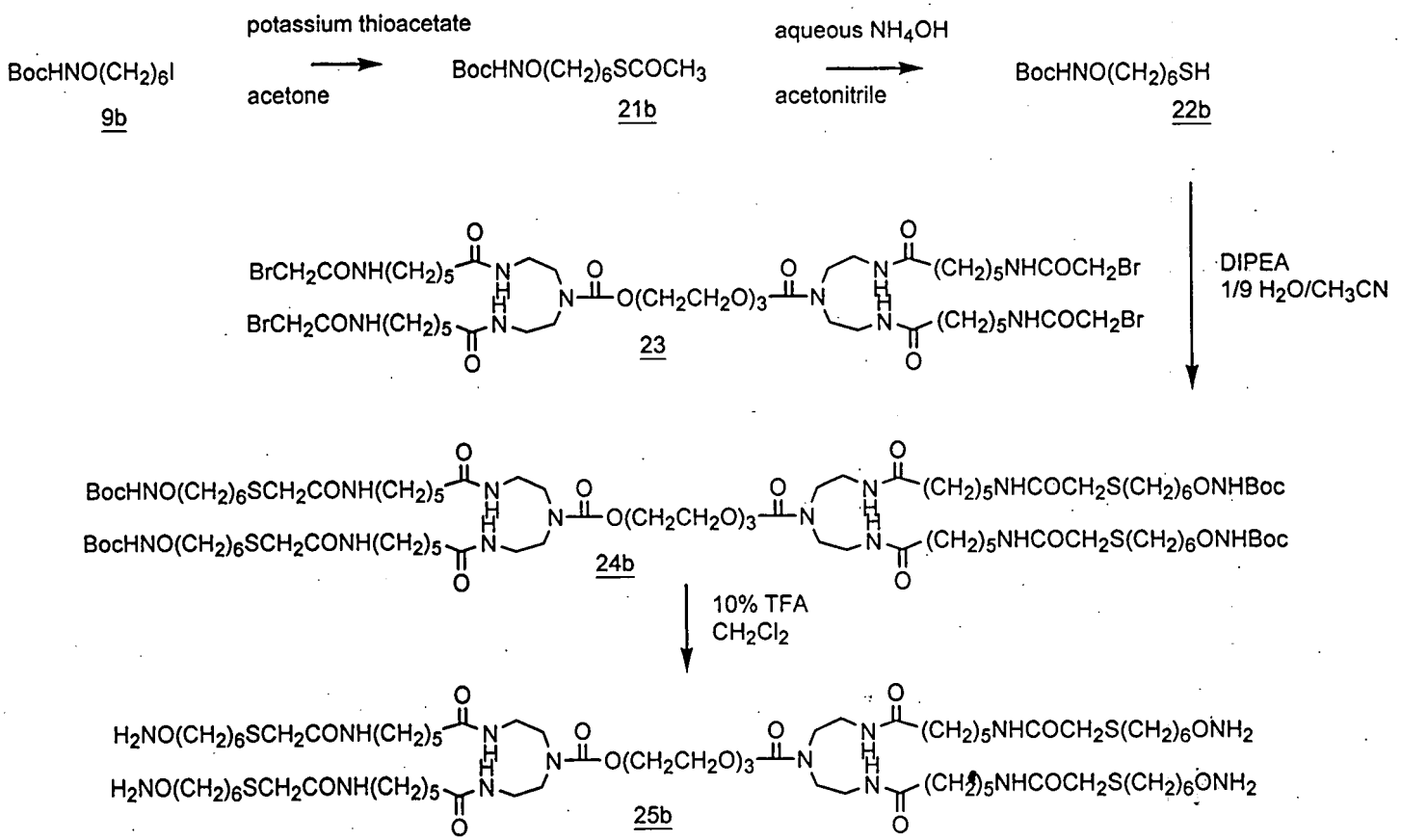

potassium thioacetate
acetone
aqueous NH4OH
acetonitrile
BocHNO(CH2)6I
9b
BocHNO(CH2)6SCOCH3
21b
BocHNO(CH2)6SH
22b
BrCH2CONH(CH2)5
O(CH2CH2O)3
(CH2)5NHCOCH2Br
23
DIPEA
1/9 H2O/CH3CN
BocHNO(CH2)6SCH2CONH(CH2)5
(CH2)5NHCOCH2S(CH2)6ONHBoc
24b
10% TFA
CH2Cl2
H2NO(CH2)6SCH2CONH(CH2)5
(CH2)5NHCOCH2S(CH2)6ONH2
25b

Figure 6

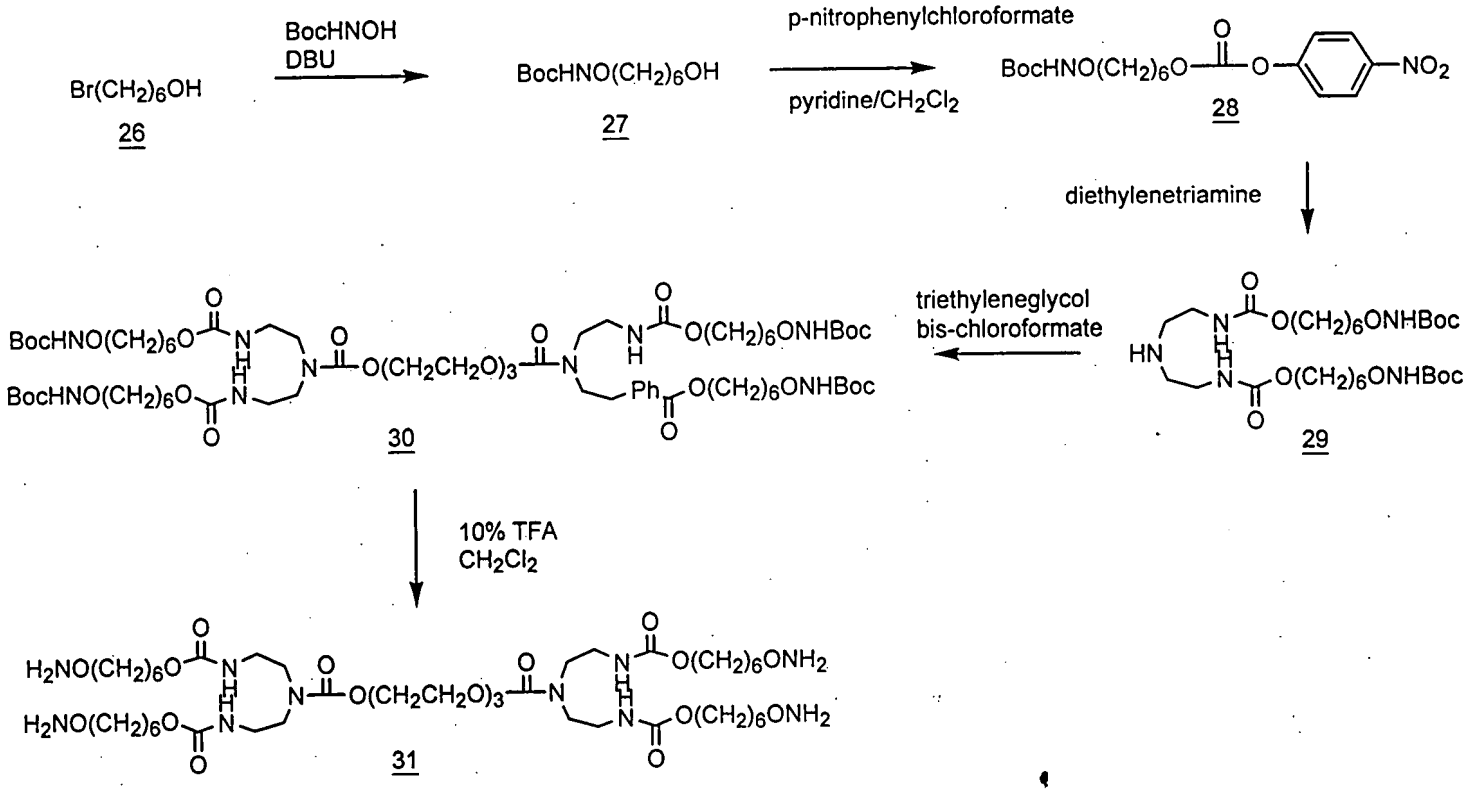

Br(CH2)6OH
26
BocHNOH
DBU
BocHNO(CH2)6OH
27
p-nitrophenylchloroformate
pyridine/CH2Cl2
BocHNO(CH2)6O
O
O
NO2
28
diethylenetriamine
triethyleneglycol
bis-chloroformate
HN
O(CH2)6ONHBoc
O(CH2)6ONHBoc
29
BocHNO(CH2)6O
BocHNO(CH2)6O
O(CH2CH2O)3
Ph
O(CH2)6ONHBoc
O(CH2)6ONHBoc
30
10% TFA
CH2Cl2
H2NO(CH2)6O
H2NO(CH2)6O
O(CH2CH2O)3
O(CH2)6ONH2
O(CH2)6ONH2
31

Figure 7

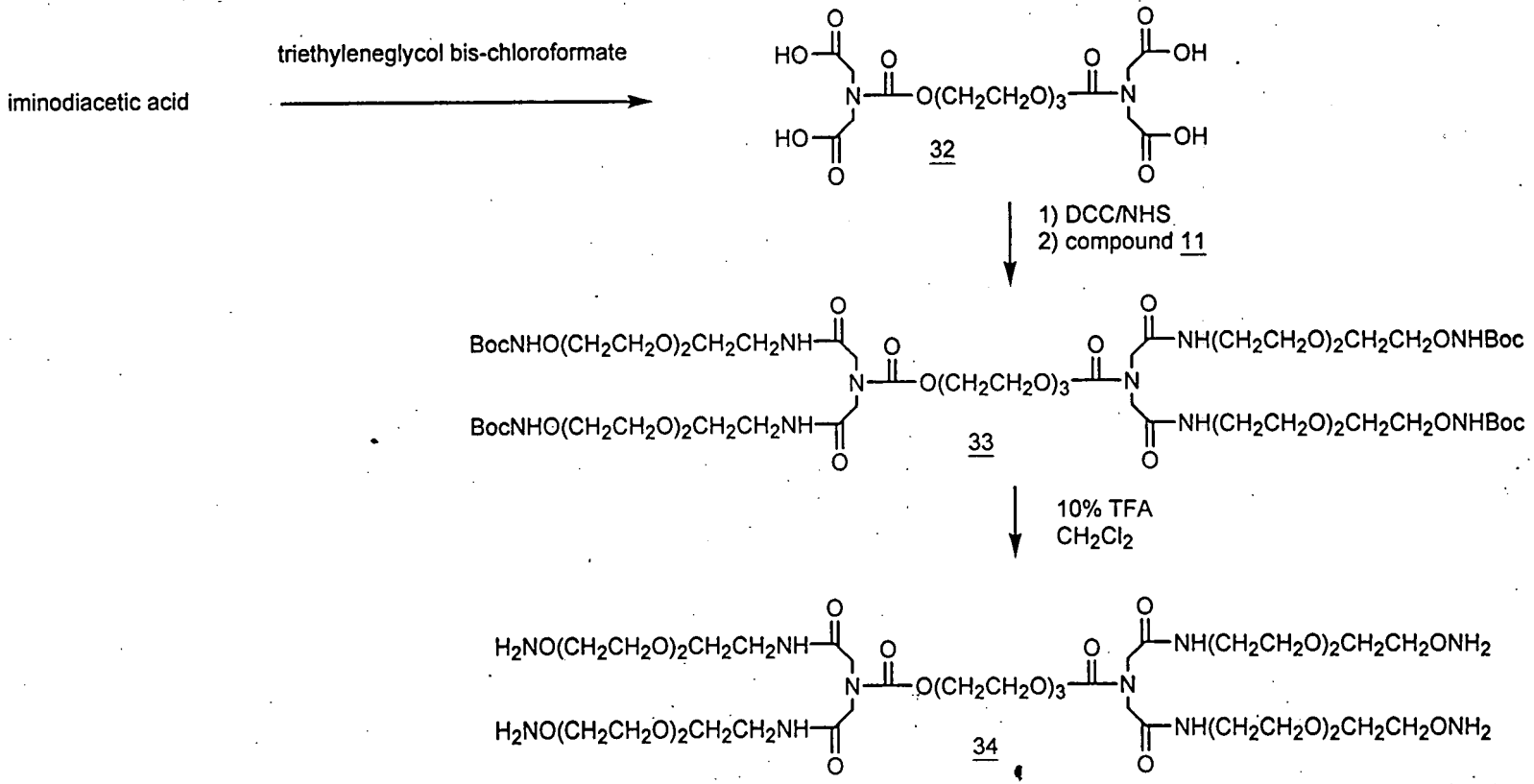

iminodiacetic acid
triethyleneglycol bis-chloroformate
32
1) DCC/NHS
2) compound 11
33
10% TFA
CH_2Cl_2
34

Figure 8

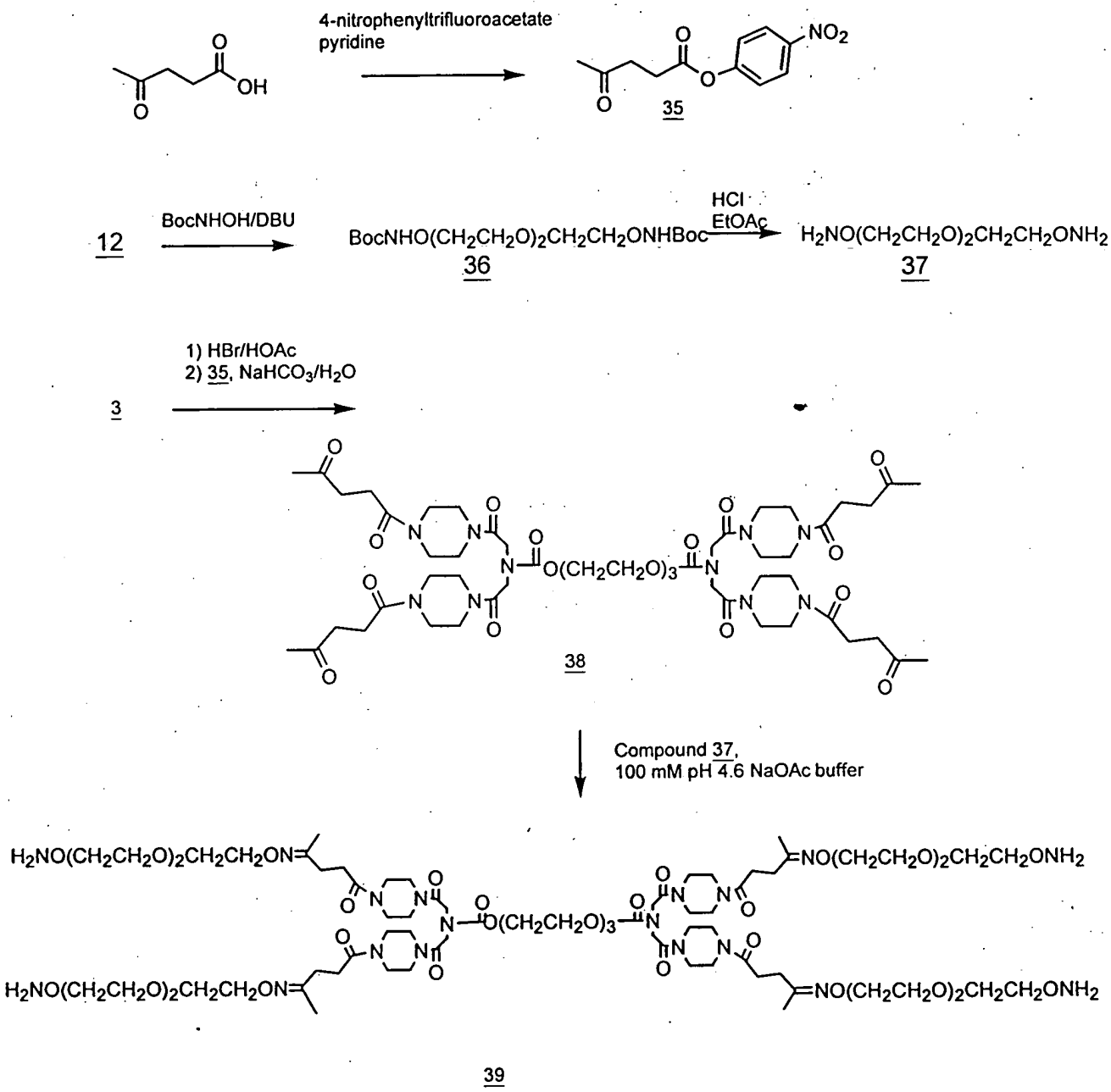

4-nitrophenyltrifluoroacetate
pyridine
35
12
BocNHOH/DBU
BocNHO(CH2CH2O)2CH2CH2ONHBoc
36
HCl
EtOAc
H2NO(CH2CH2O)2CH2CH2ONH2
37
1) HBr/HOAc
2) 35, NaHCO3/H2O
3
38
Compound 37,
100 mM pH 4.6 NaOAc buffer
H2NO(CH2CH2O)2CH2CH2ON=
NO(CH2CH2O)2CH2CH2ONH2
39

Figure 9

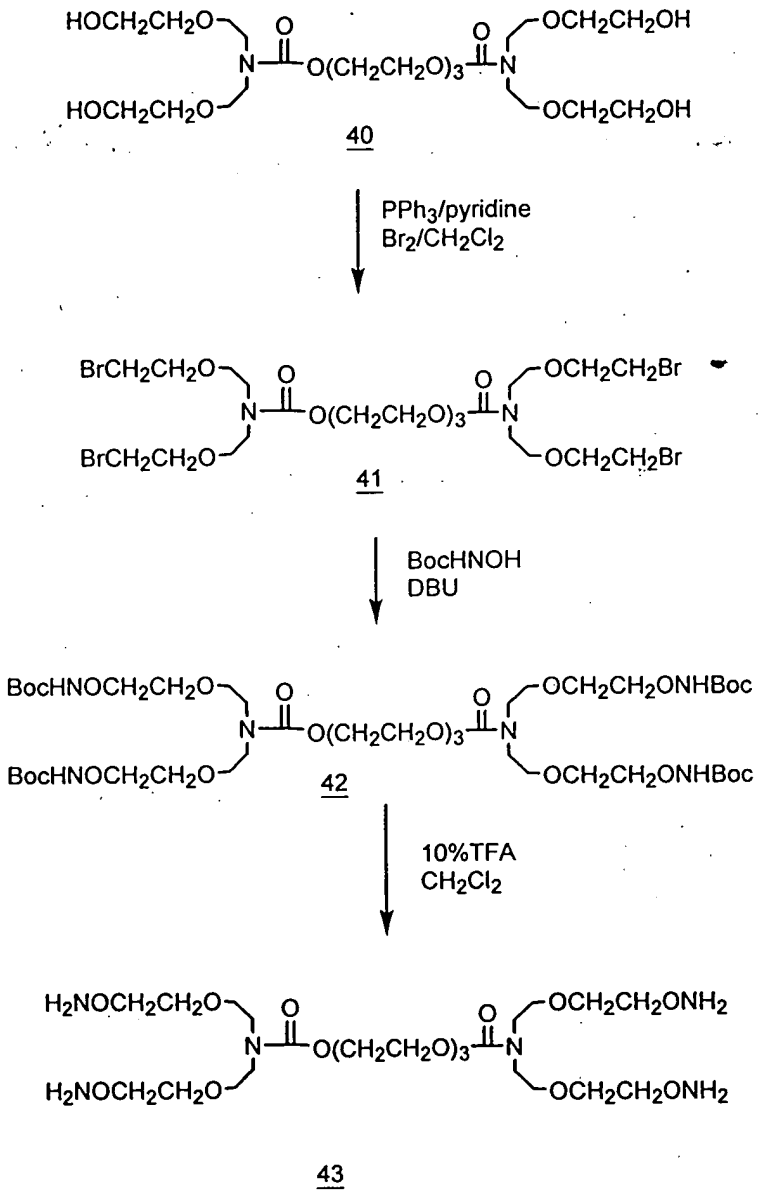

HOCH2CH2O
HOCH2CH2O
O
N
O(CH2CH2O)3
OCH2CH2OH
OCH2CH2OH
40
PPh3/pyridine
Br2/CH2Cl2
BrCH2CH2O
BrCH2CH2O
OCH2CH2Br
OCH2CH2Br
41
BocHNOH
DBU
BocHNOCH2CH2O
BocHNOCH2CH2O
OCH2CH2ONHBoc
OCH2CH2ONHBoc
42
10%TFA
CH2Cl2
H2NOCH2CH2O
H2NOCH2CH2O
OCH2CH2ONH2
OCH2CH2ONH2
43

Figure 10

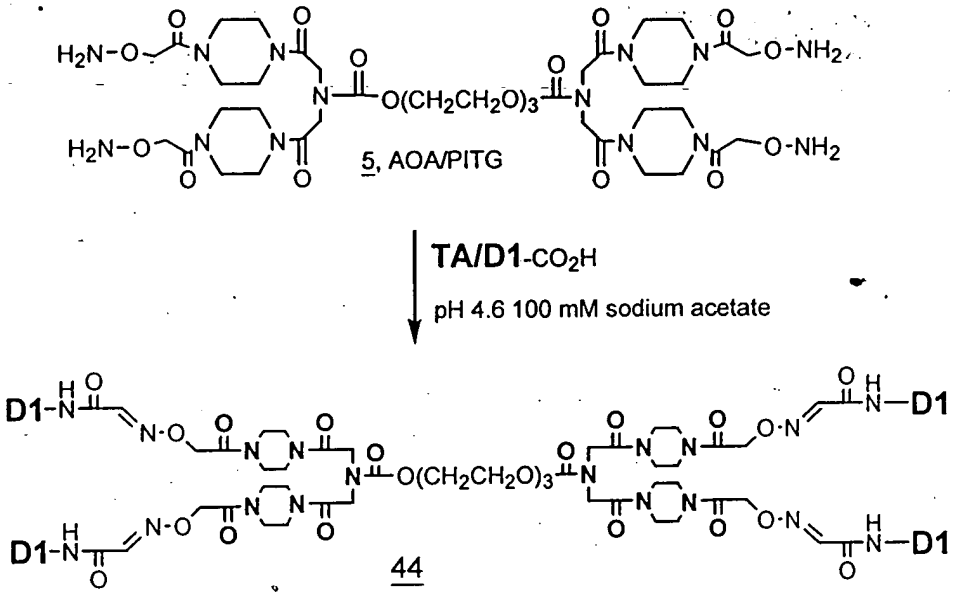
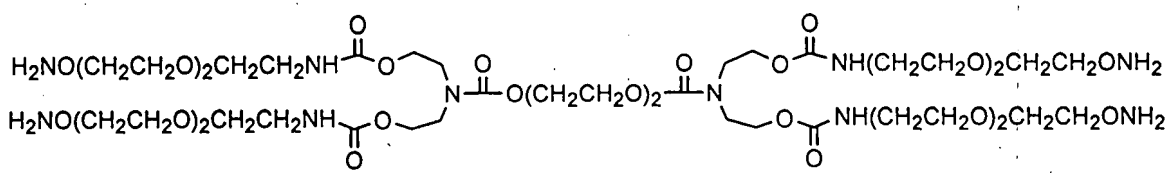
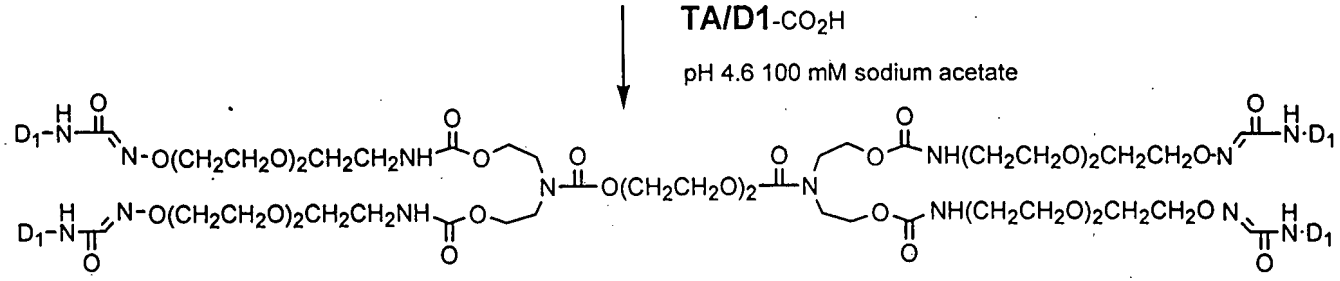


Figure 11



Compound 16, AOTEG/DEA/DEG Platform



45

Figure 12

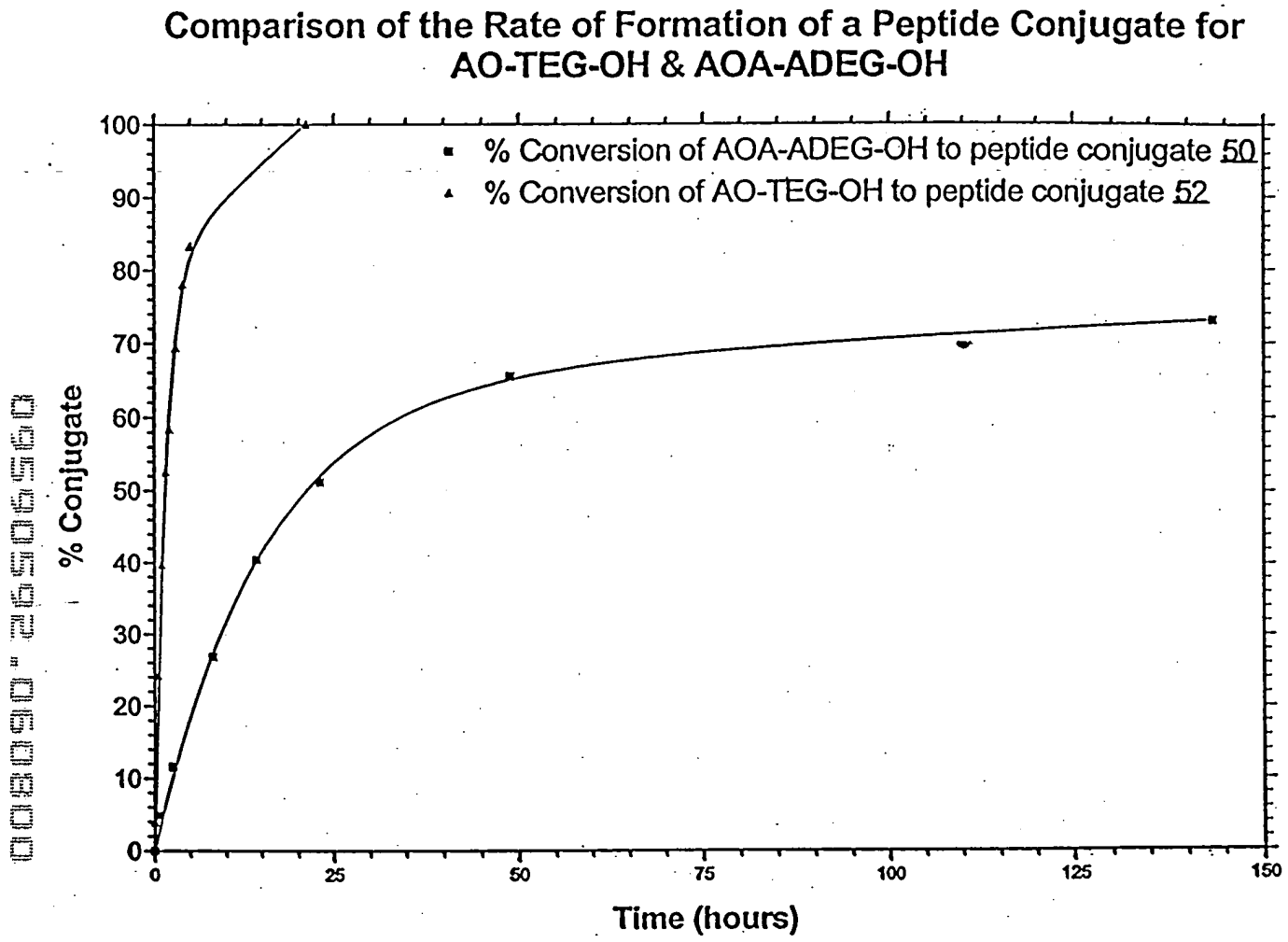

Comparison of the Rate of Formation of a Peptide Conjugate for AO-TEG-OH & AOA-ADEG-OH
% Conversion of AOA-ADEG-OH to peptide conjugate 50
% Conversion of AO-TEG-OH to peptide conjugate 52
% Conjugate
Time (hours)
0
10
20
30
40
50
60
70
80
90
100
0
25
50
75
100
125
150

Figure 13

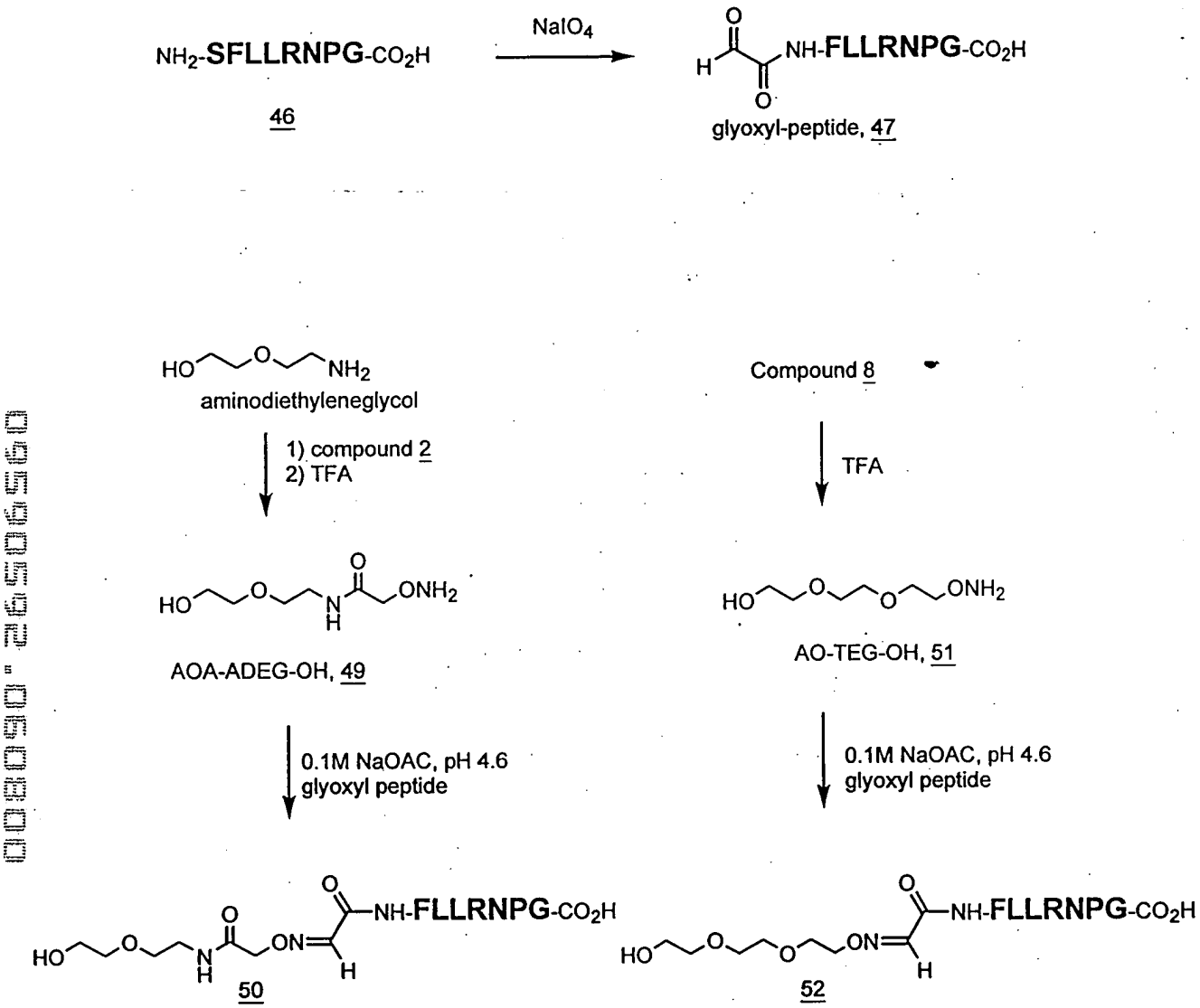

NH2-SFLLRNPG-CO2H
46
NaIO4
NH-FLLRNPG-CO2H
glyoxyl-peptide, 47
HO
O
NH2
aminodiethyleneglycol
1) compound 2
2) TFA
Compound 8
TFA
HO
O
N
H
ONH2
AOA-ADEG-OH, 49
HO
O
O
ONH2
AO-TEG-OH, 51
0.1M NaOAC, pH 4.6
glyoxyl peptide
0.1M NaOAC, pH 4.6
glyoxyl peptide
NH-FLLRNPG-CO2H
50
52

Figure 14

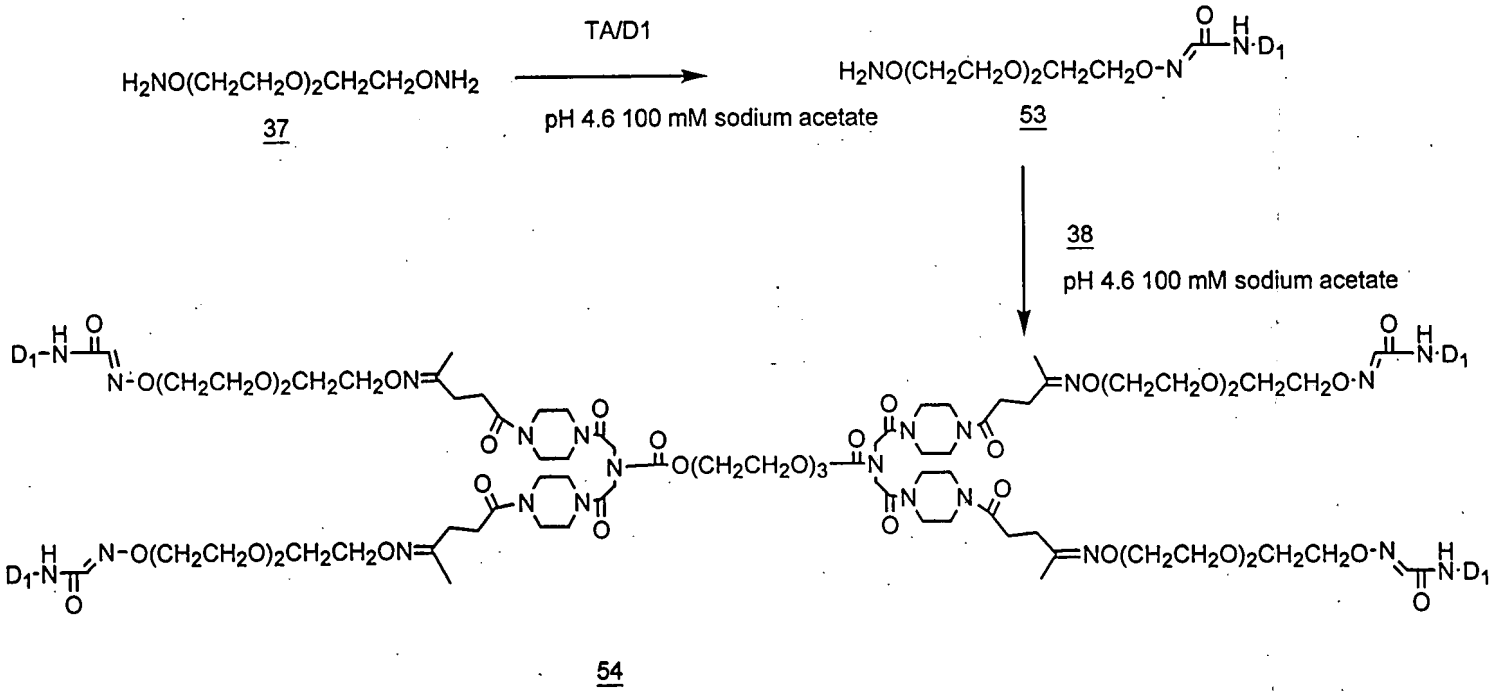

TA/D1
H2NO(CH2CH2O)2CH2CH2ONH2
37
pH 4.6 100 mM sodium acetate
H2NO(CH2CH2O)2CH2CH2O-N
N·D1
53
38
pH 4.6 100 mM sodium acetate
D1-N
N-O(CH2CH2O)2CH2CH2ON=
O(CH2CH2O)3
=NO(CH2CH2O)2CH2CH2O-N
54

Figure 15

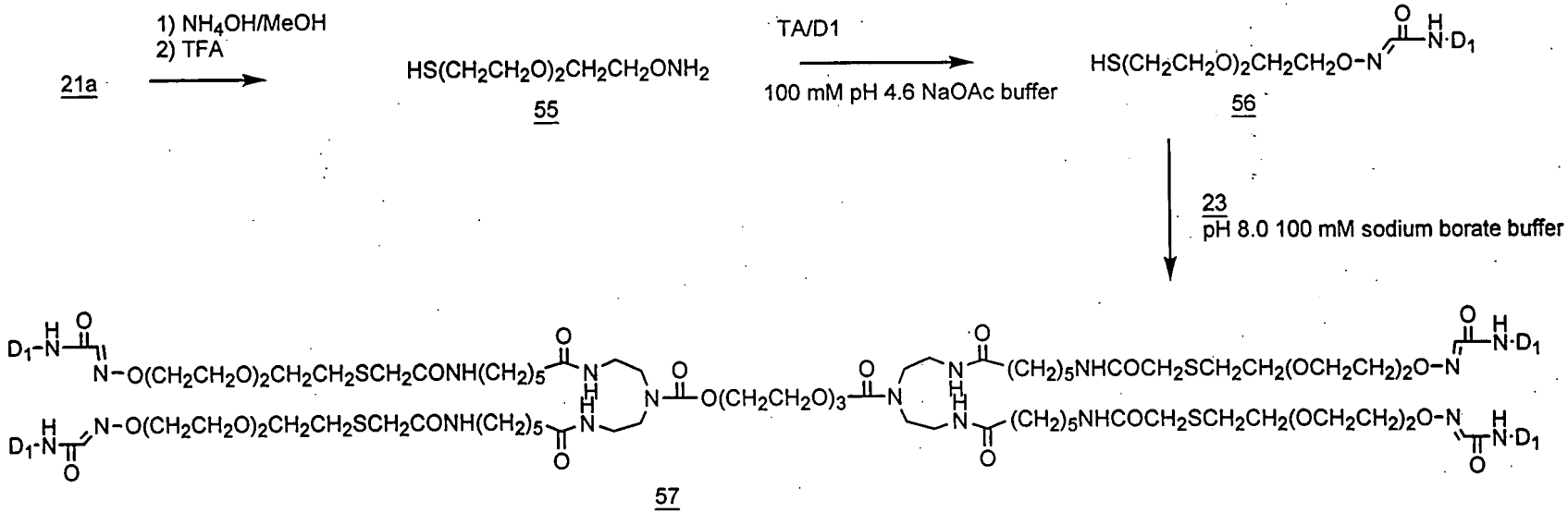
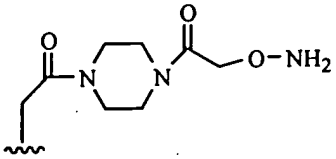
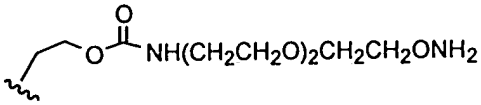
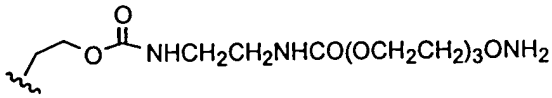
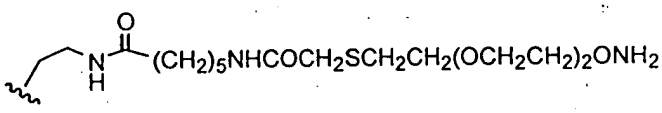

1) NH4OH/MeOH
2) TFA
21a
HS(CH2CH2O)2CH2CH2ONH2
55
TA/D1
100 mM pH 4.6 NaOAc buffer
HS(CH2CH2O)2CH2CH2O-N
56
23
pH 8.0 100 mM sodium borate buffer
57

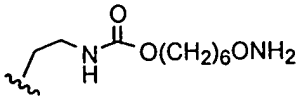
Figure 16

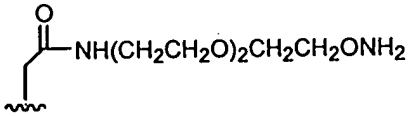


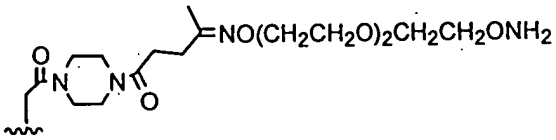












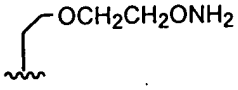
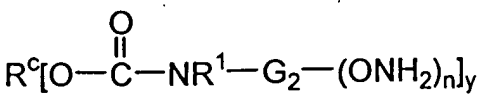
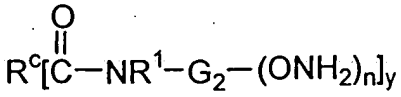


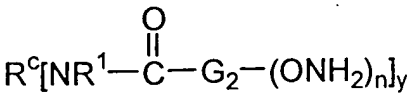
Figure 17



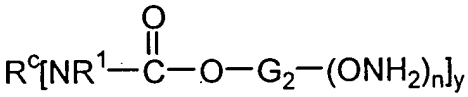
Formula 3



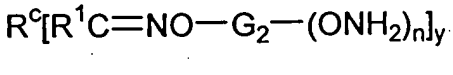
Formula 4



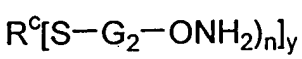
Formula 5



Formula 6

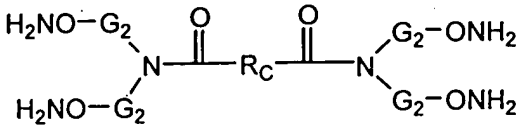


Formula 7

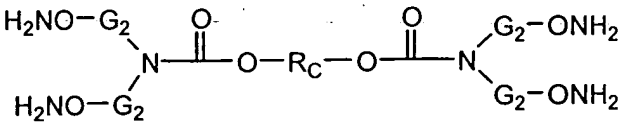


Formula 8

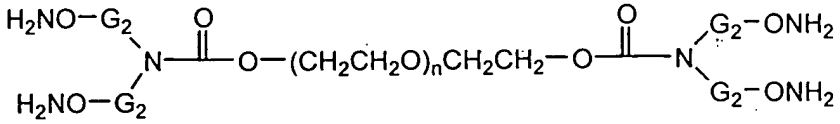
Figure 18



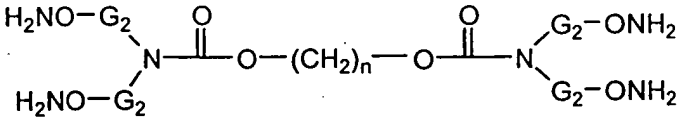
Formula 9



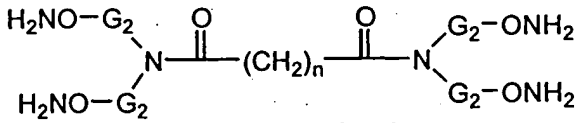
Formula 10



Formula 11

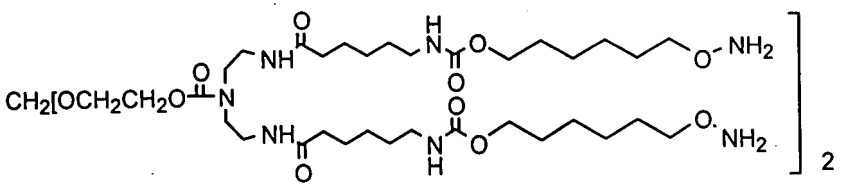


Formula 12

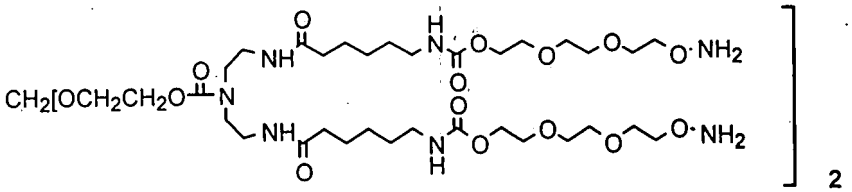


Formula 13

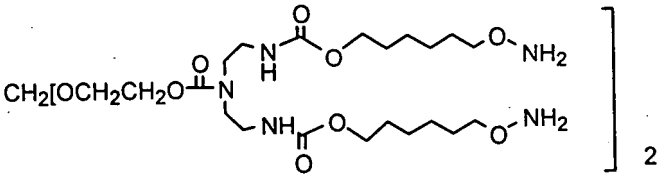
Figure 19



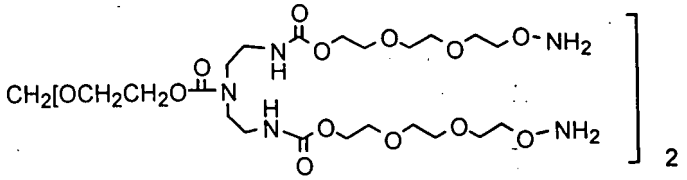
80



81

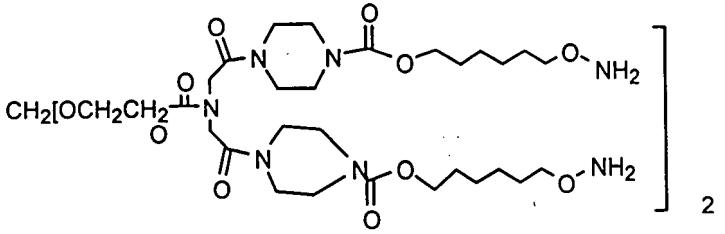


82

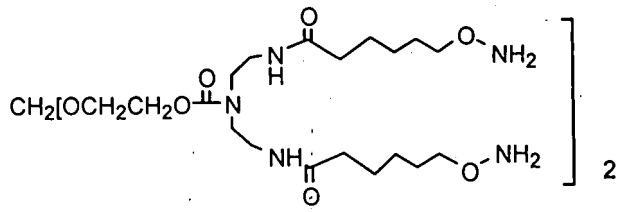


83

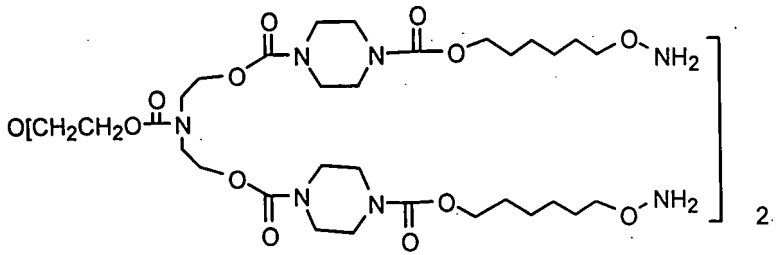
Figure 20



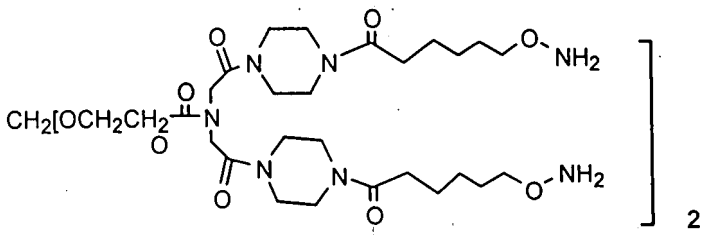
84



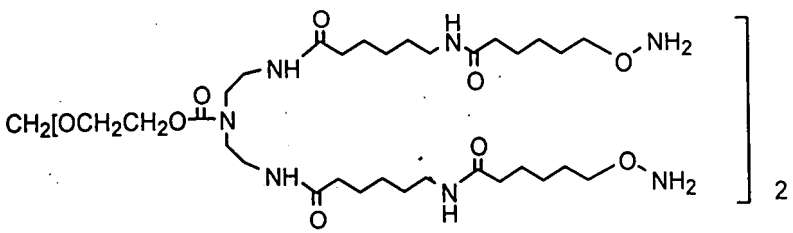
87



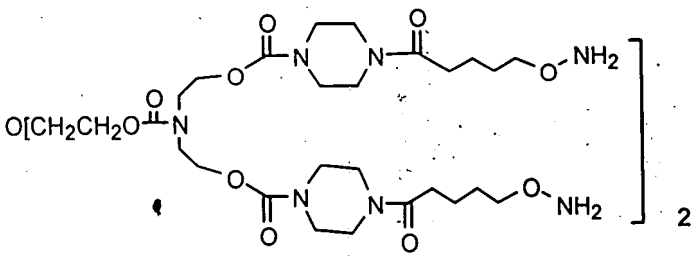
85



88

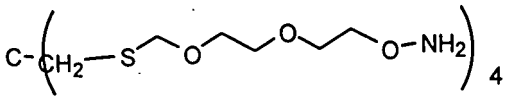


86

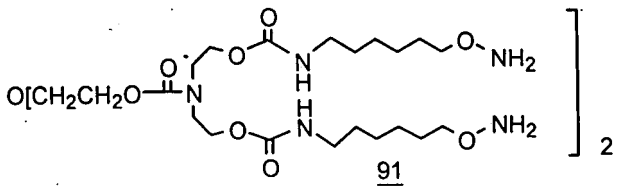


89

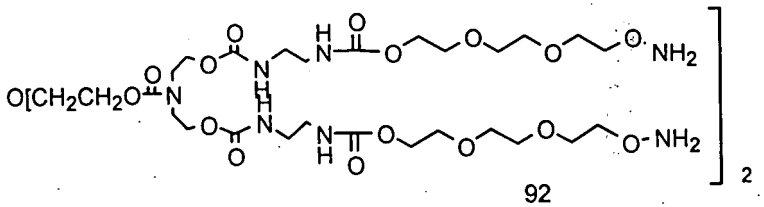
Figure 21



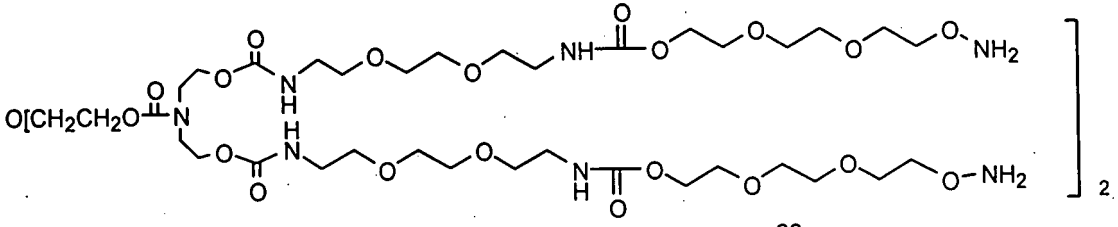
90



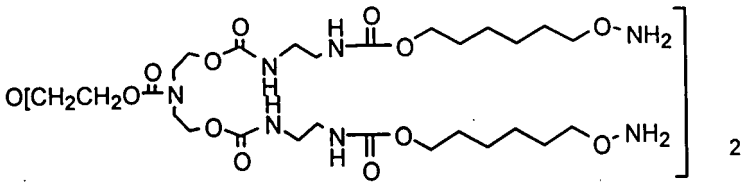
91



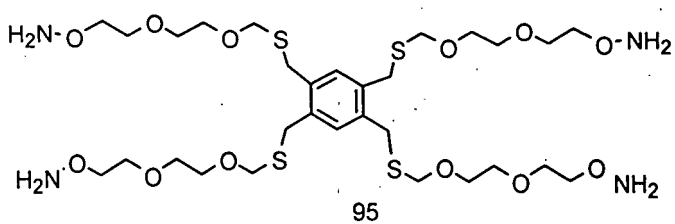
92



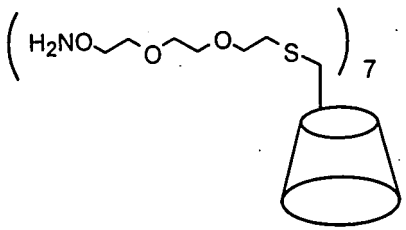
93



94

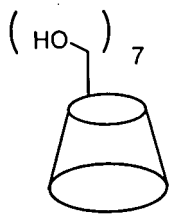


95



96

Where



97

= beta-cyclodextrin

Figure 22

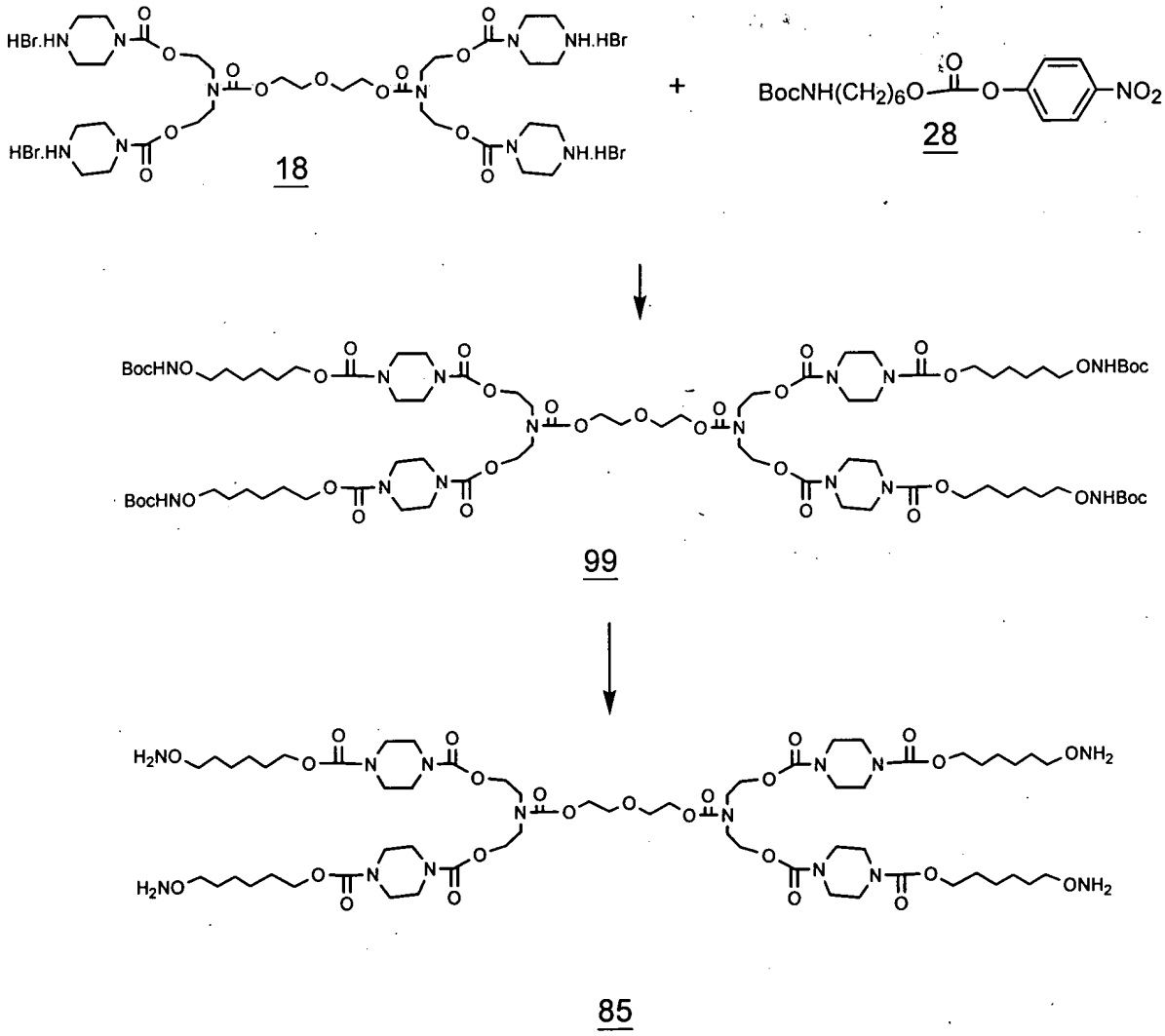

HBr.HN
NH.HBr
18
+
BocNH(CH2)6O
NO2
28
BocHNO
ONHBoc
99
H2NO
ONH2
85

Figure 23

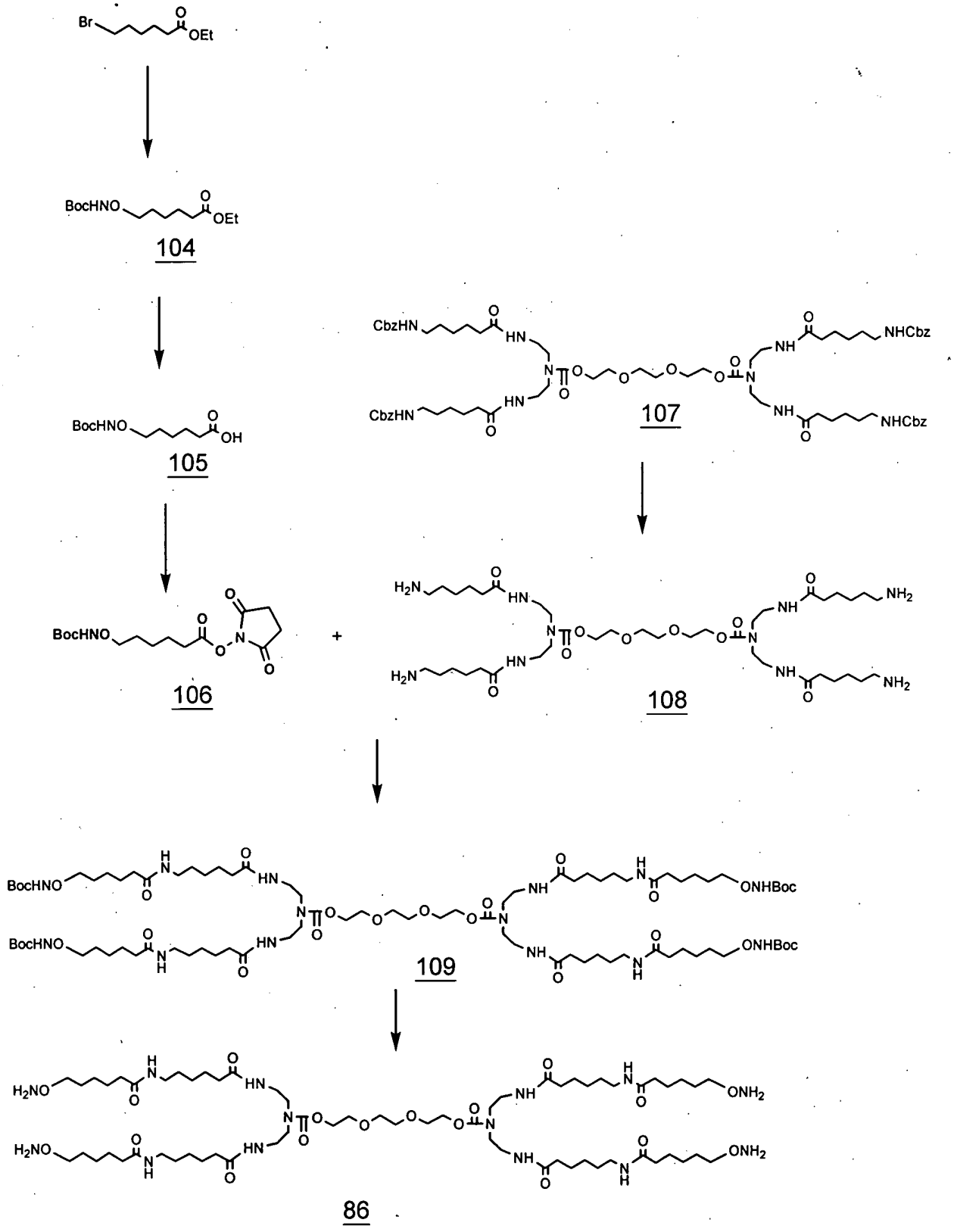

Br
OEt
BocHNO
OEt
104
BocHNO
OH
105
CbzHN
HN
NH
NHCbz
107
BocHNO
106
+
H_2N
NH
NH_2
108
BocHNO
ONHBoc
109
H_2NO
ONH_2
86

Figure 24

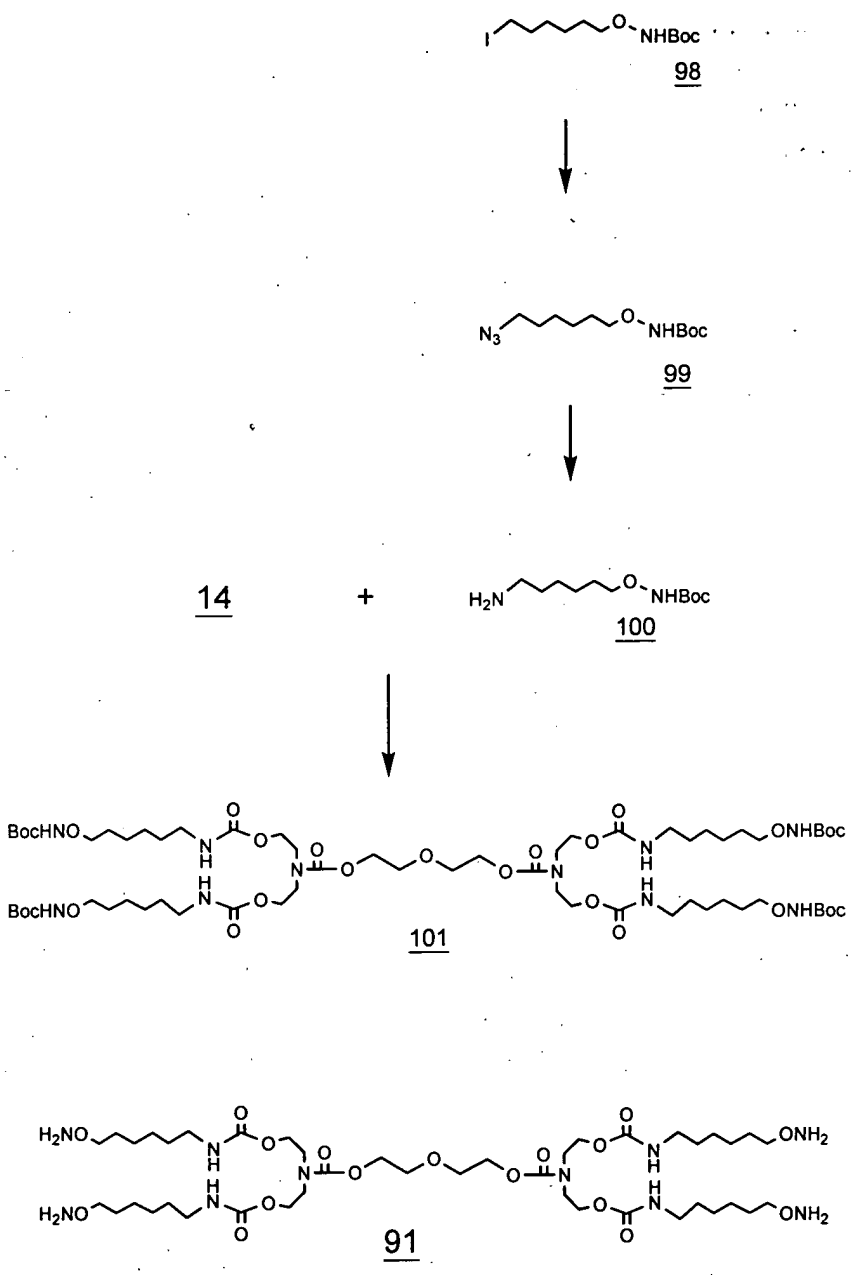

NHBoc
98
N3
NHBoc
99
14
+
H2N
NHBoc
100
BocHNO
BocHNO
ONHBoc
ONHBoc
101
H2NO
H2NO
ONH2
ONH2
91

Figure 25

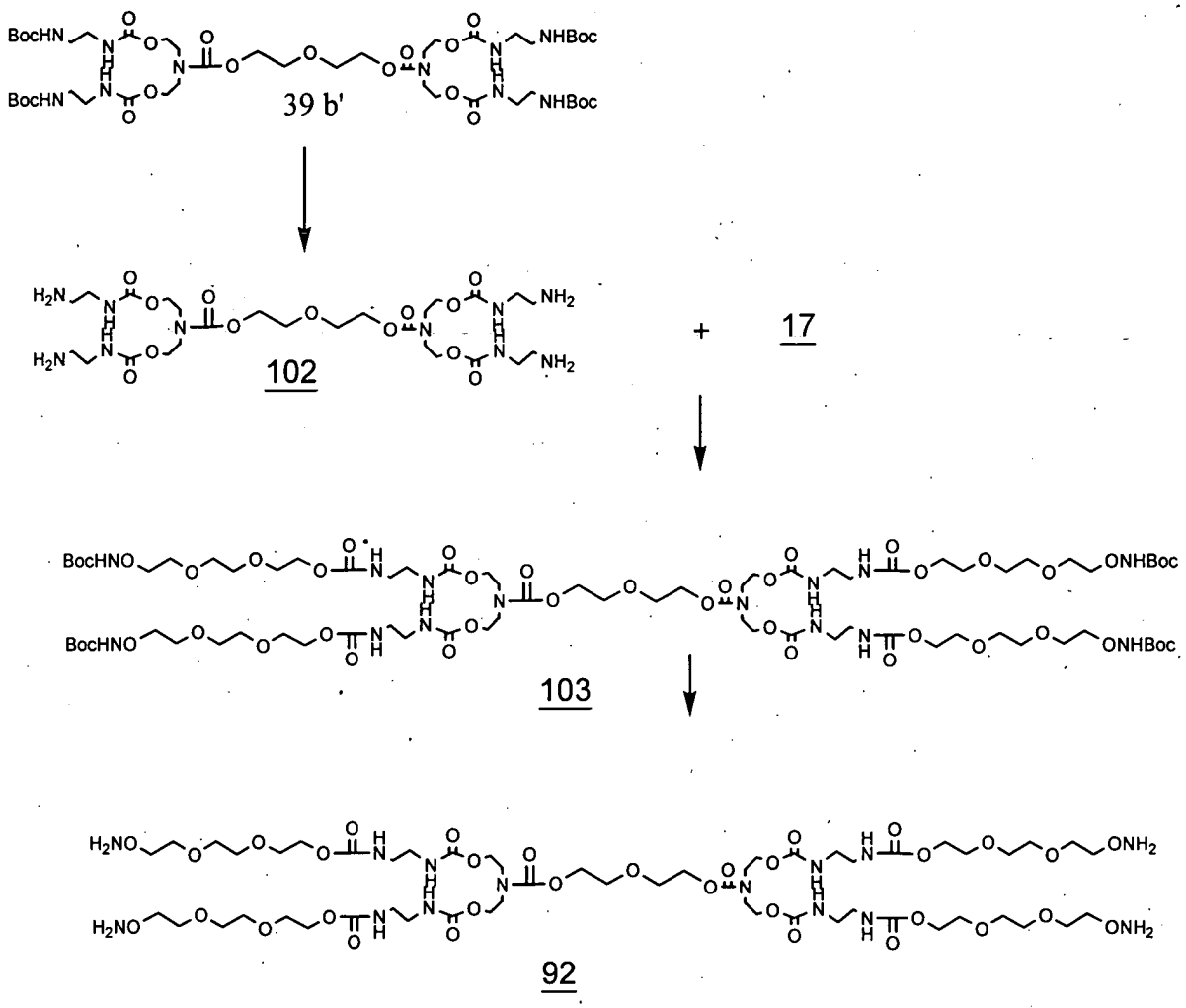

BocHN
NHBoc
BocHN
NHBoc
39 b'
H_2N
NH_2
H_2N
NH_2
102
+
17
BocHNO
ONHBoc
BocHNO
ONHBoc
103
H_2NO
ONH_2
H_2NO
ONH_2
92

Figure 26

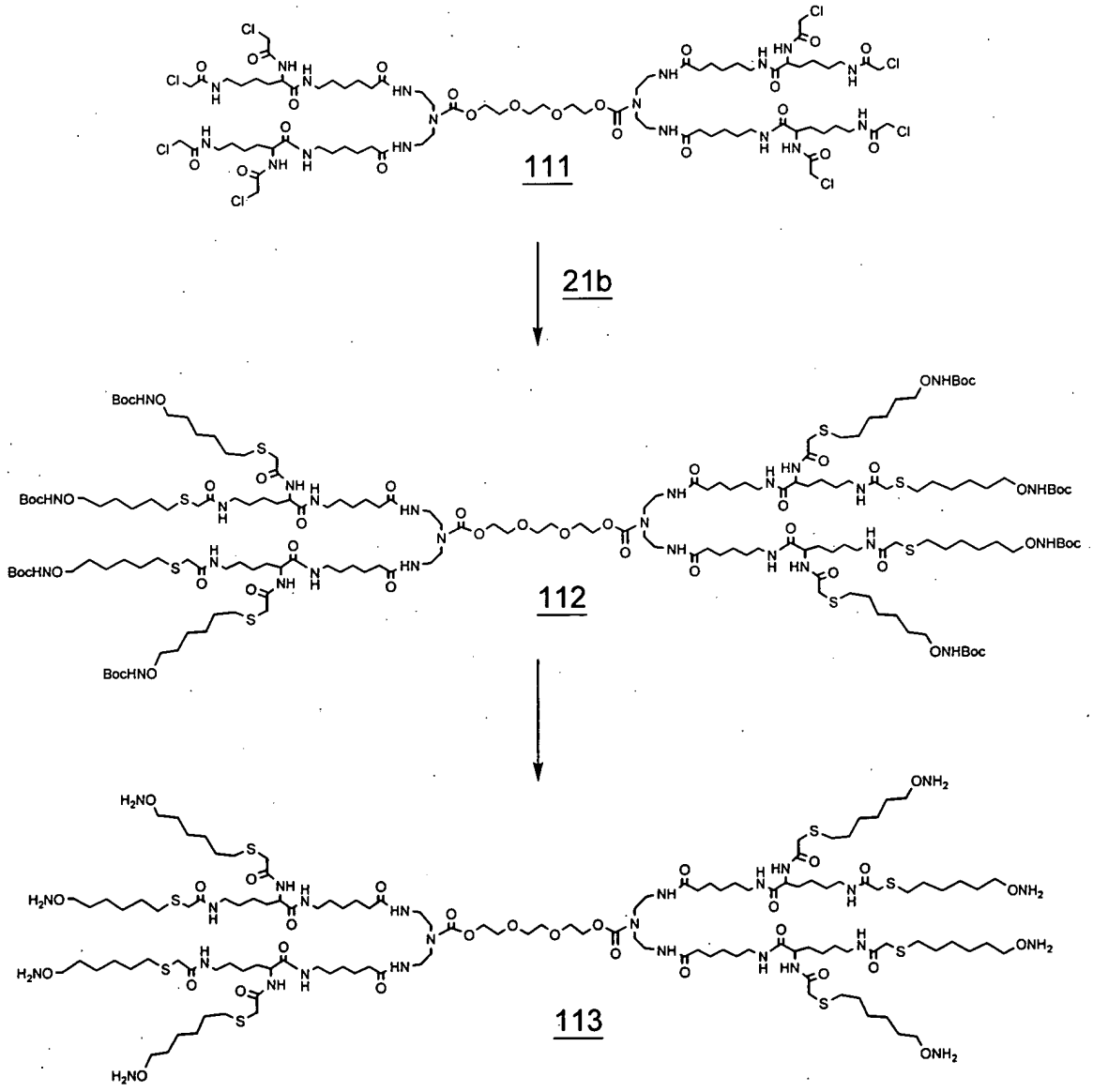


Figure 27

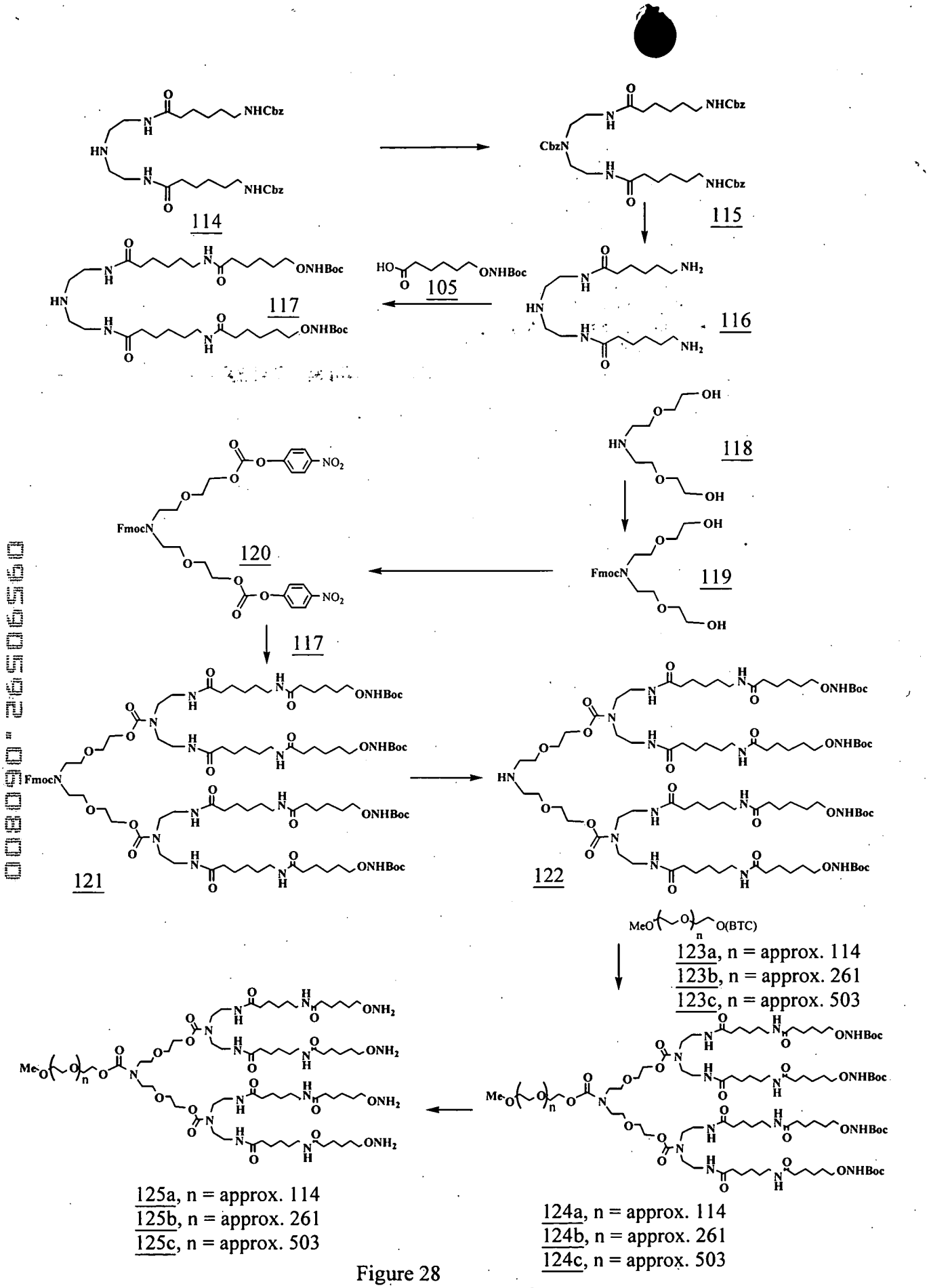

NHCbz
114
115
116
117
105
ONHBoc
118
119
120
121
122
123a, n = approx. 114
123b, n = approx. 261
123c, n = approx. 503
124a, n = approx. 114
124b, n = approx. 261
124c, n = approx. 503
125a, n = approx. 114
125b, n = approx. 261
125c, n = approx. 503

Figure 28

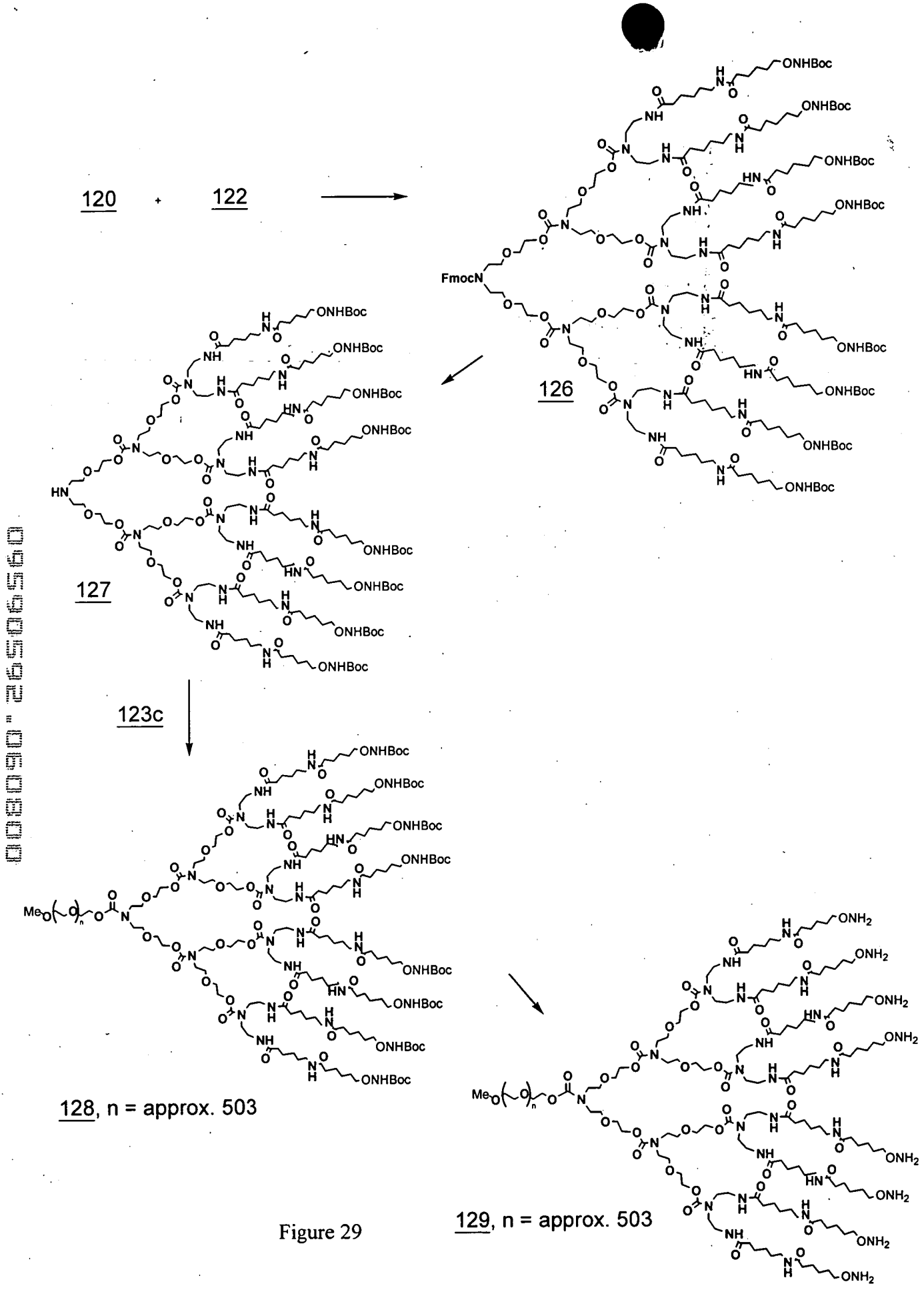


Figure 29

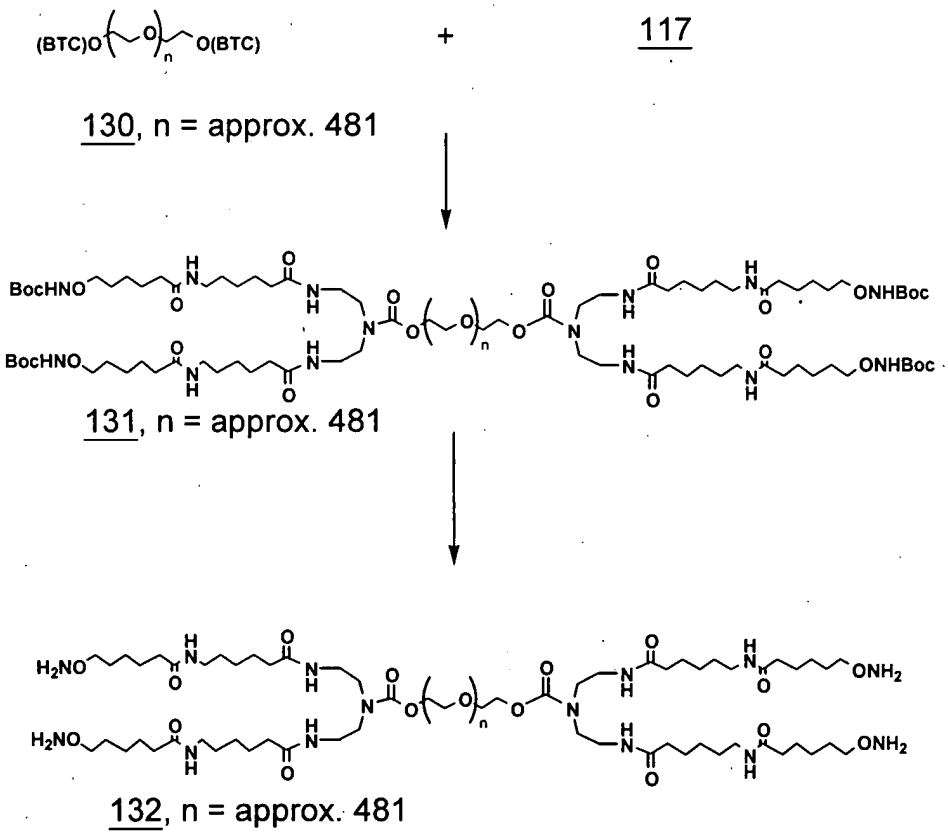

(BTC)O
O(BTC)
+
117
130, n = approx. 481
BocHNO
ONHBoc
131, n = approx. 481
H2NO
ONH2
132, n = approx. 481

Figure 30

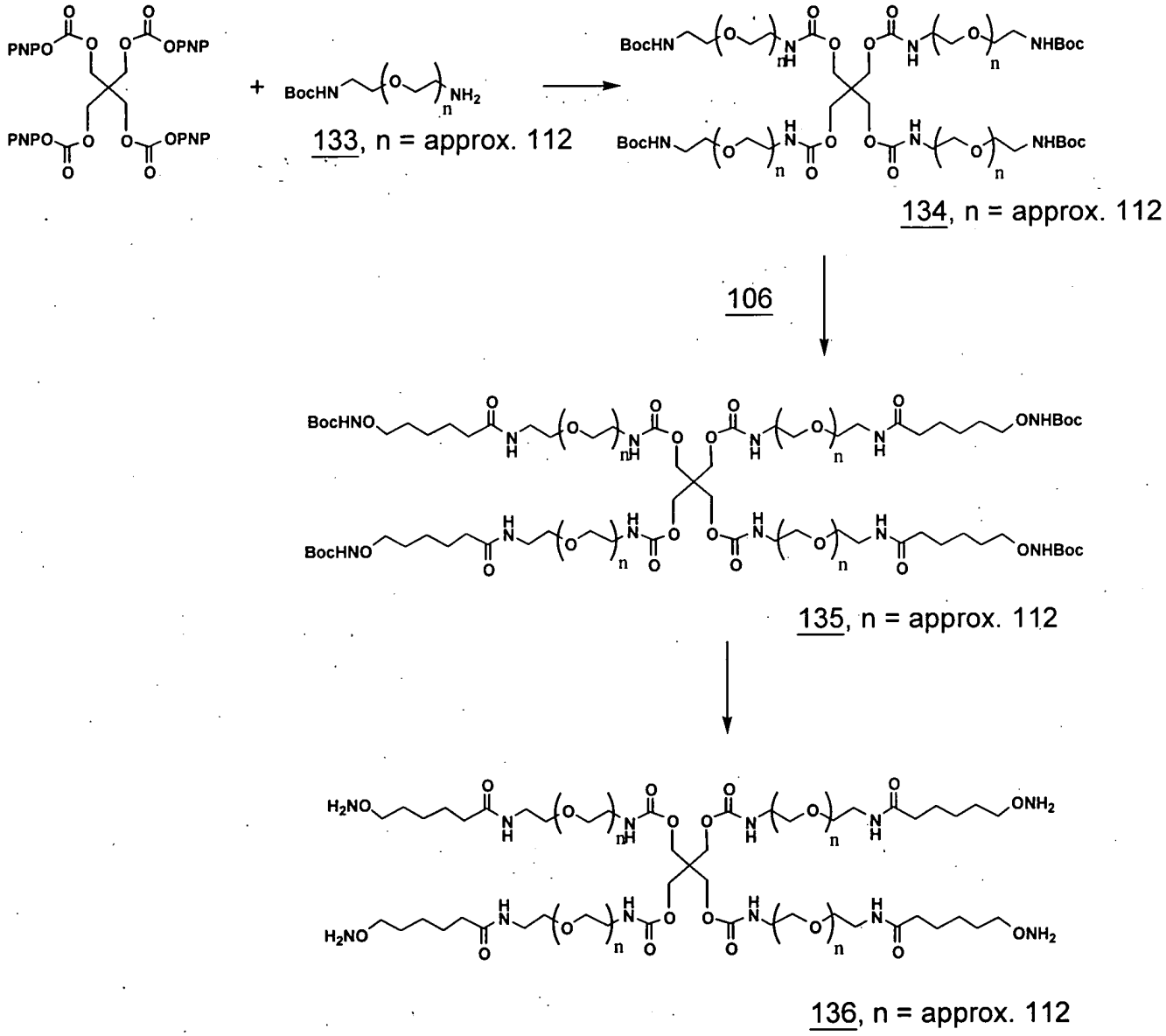

PNPO
OPNP
PNPO
OPNP
BocHN
NH2
133, n = approx. 112
BocHN
NHBoc
134, n = approx. 112
106
BocHNO
ONHBoc
135, n = approx. 112
H2NO
ONH2
136, n = approx. 112

Figure 31

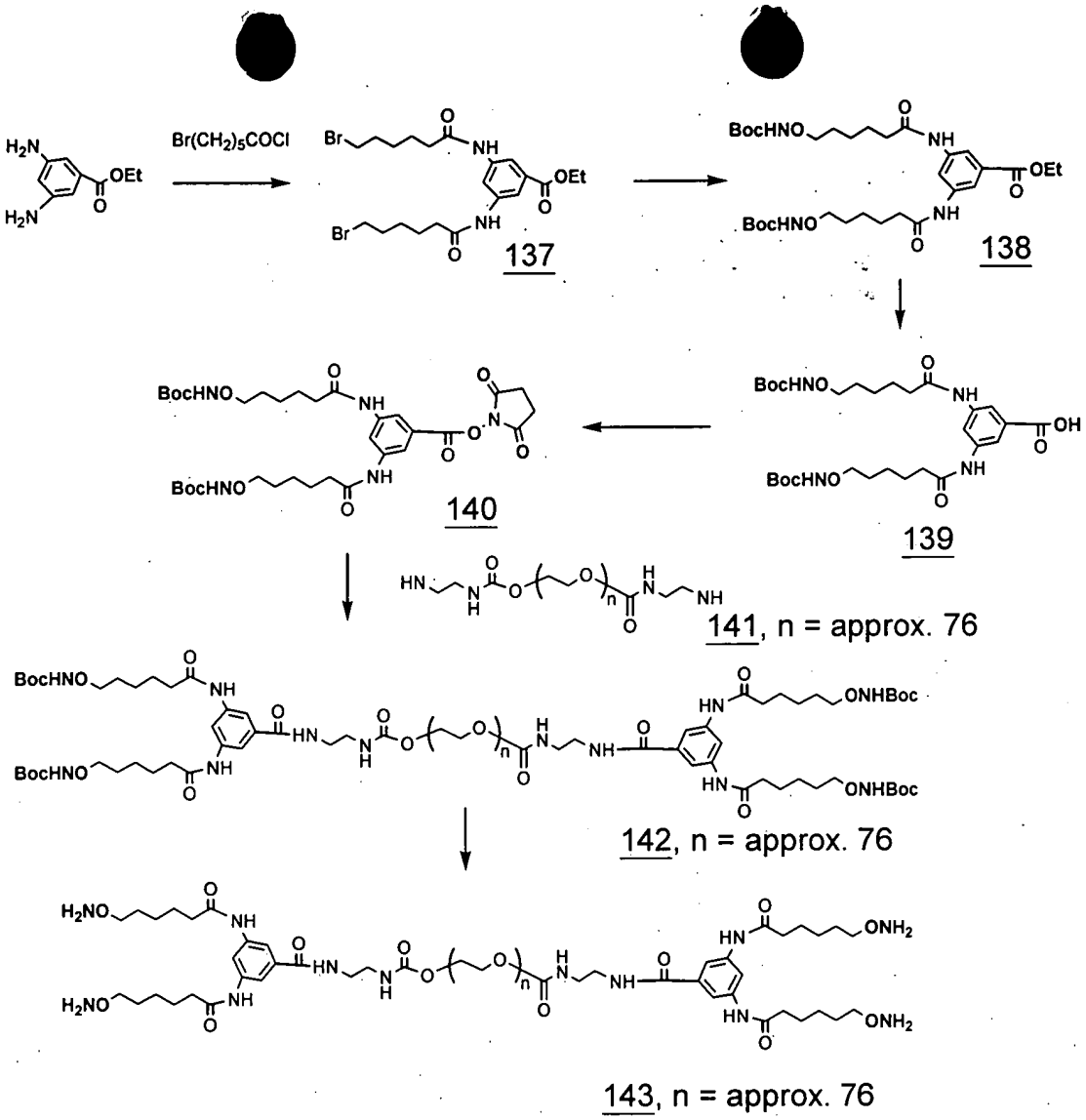
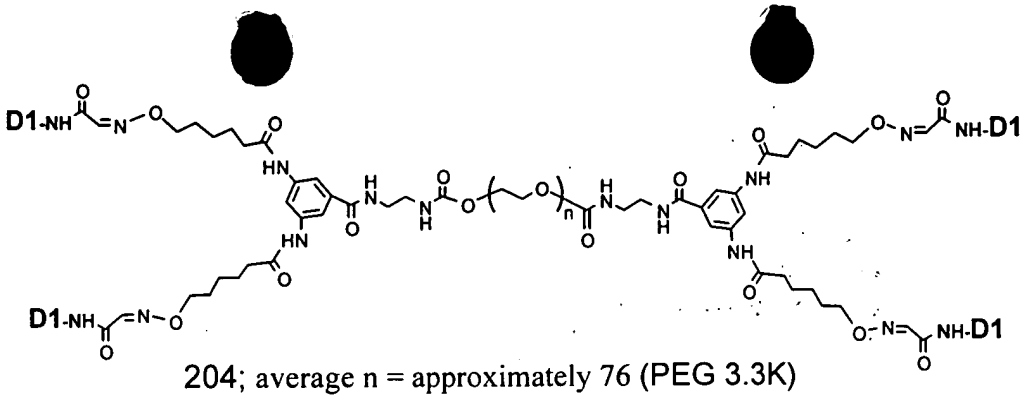
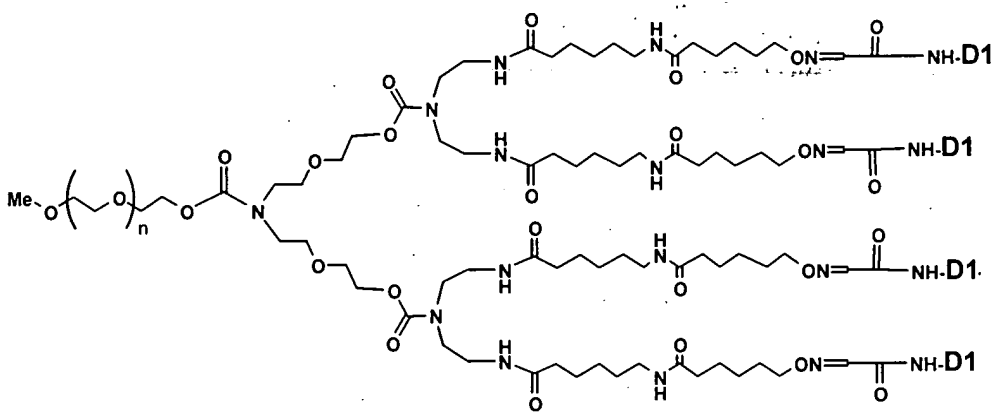

Br(CH2)5COCl
137
138
139
140
141, n = approx. 76
142, n = approx. 76
143, n = approx. 76

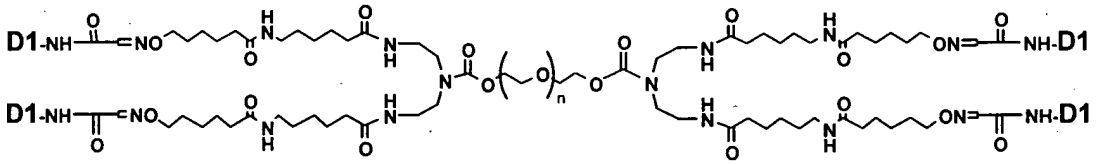
Figure 32



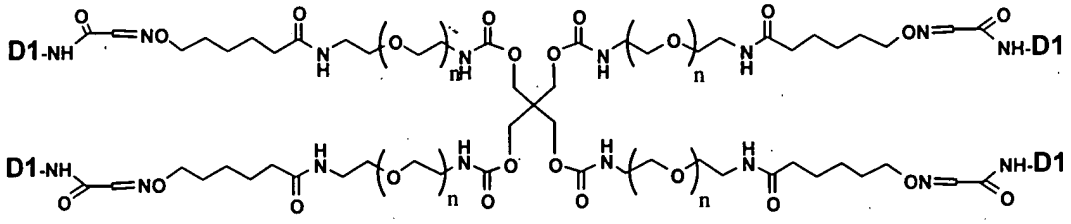
204; average n = approximately 76 (PEG 3.3K)



200; average n = approximately 503 (PEG 20K)
201; average n = approximately 114 (PEG 5K)
205; average n = approximately 261 (PEG 12K)



202; average n = approximately 503 (PEG 20K)



203; average n = approximately 125 (PEG 5K)
total PEG = 20K

Figure 33

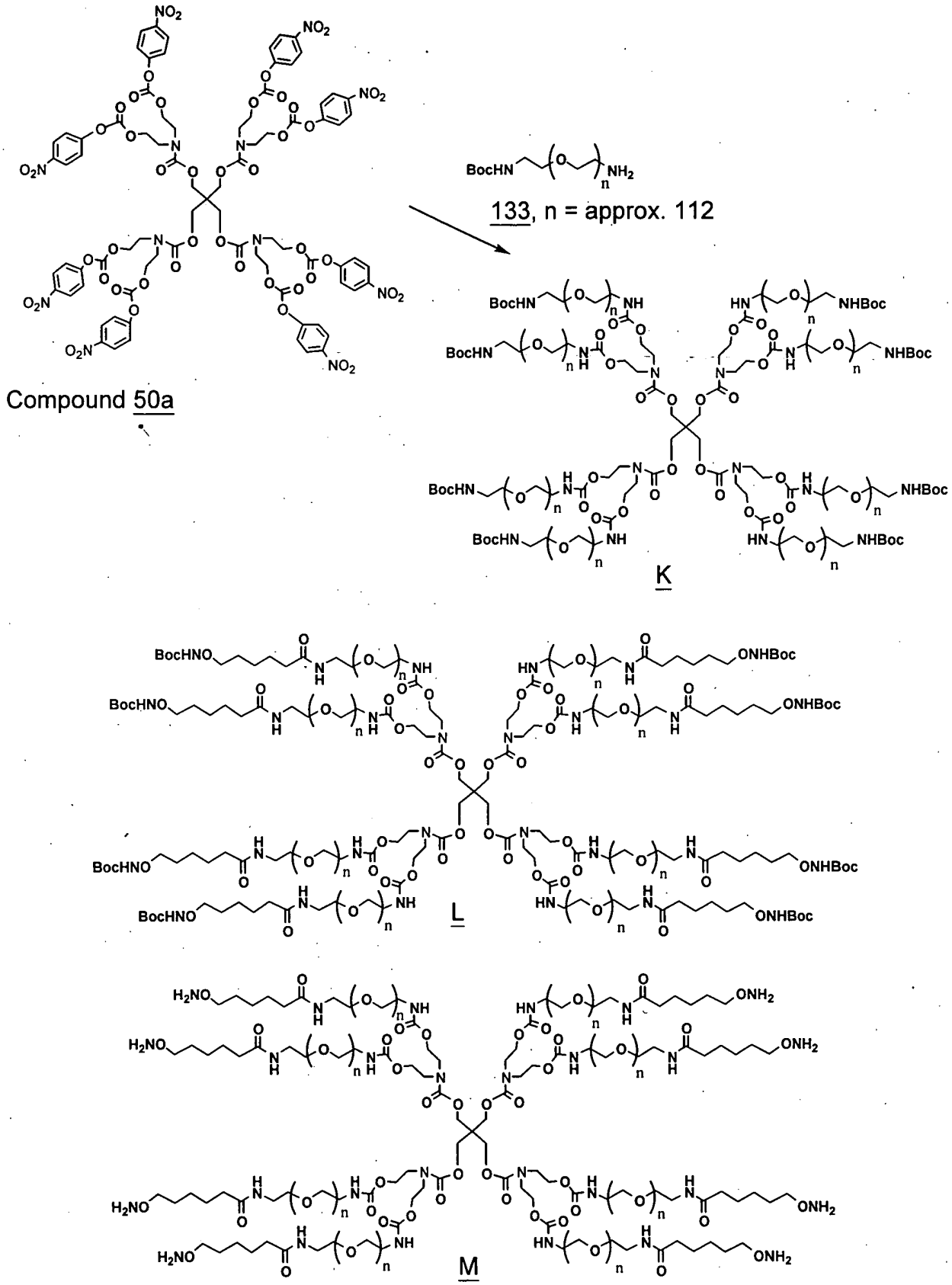
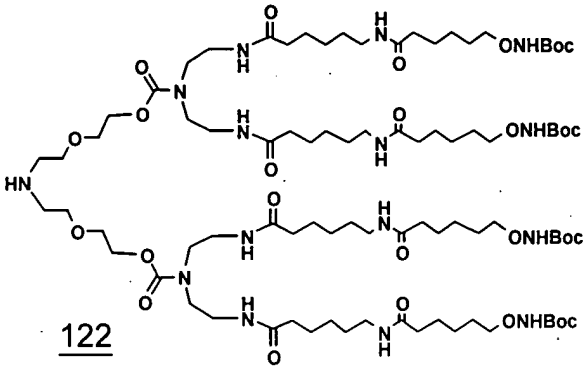
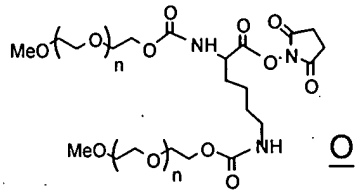


Figure 34

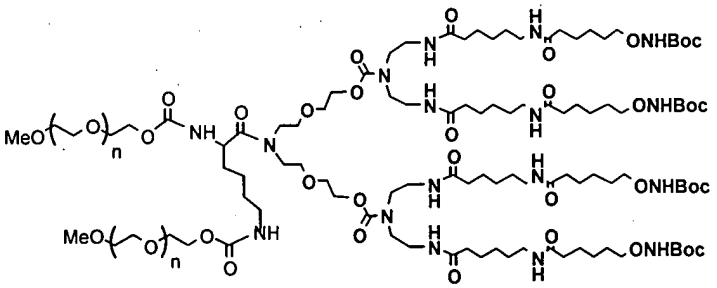


122



pyridine

O



P

Figure 35